ASPECTS OF OVIPOSITION IN THE FOWL

by

A. H. SYKES
Poultry Research Centre
Edinburgh.

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CONTENTS

INTRODUCTION

Each part of the hen's oviduct possesses to some extent, the properties of secretion and motility. Those secretions of the oviduct which contribute to egg formation have been studied by several authors but the mechanics of egg formation have so far received little attention.

The oviduct may be considered to show two types of movement: relatively slow, propulsive movements by means of which the egg is moved as far as the uterus, and relatively quick, expulsive movements which constitute the process of oviposition. The object of this work is to describe some aspects of the physiology of these latter movements, to suggest possible ways in which they are controlled and to compare them with similar movements observed during mammalian parturition.

THE DAILY CYCLE OF EGG PRODUCTION

Before considering in detail the present state of knowledge of oviposition it might be helpful to give a brief outline of the anatomy and physiology of egg production.

Anatomy. In the hen, as in most other birds, the reproductive apparatus is unpaired. It consists of a left ovary and a left oviduct. The oviduct of the laying hen is about 2 feet long and weighs 30 to 60 grams. It is composed of the following tissues arranged from without inwards: peritoneum, longitudinal muscle, connective tissue, circular muscle, sub-mucosa, mucosa. There is no intrinsic nerve plexus but there is a well developed extrinsic innervation arising from the lumbo-sacral cord. This innervation is, morphologically, sympathetic and parasympathetic (Hsieh, 1951) but no functional studies have been made and the sites of the nerve endings within the oviduct are unknown.

The oviduct lies in several loops suspended from the dorsal body wall by the dorsal ligament. The loops are held in position by the dorsal ligament and by the ventral ligament which hangs freely in the body cavity below the oviduct.

The oviduct is differentiated into several functional parts which may be distinguished from each other both macroscopically and microscopically. These parts are:

(1) the infundibulum or funnel, the most cranial part of the oviduct and the site of fertilization; (2) the magnum, or albumen secreting region; (3) the isthmus, where the shell membranes are secreted; (4) the uterus or shell gland a muscular sac closed at its caudal end by a sphincter muscle. The os uteri is placed asymmetrically on the ventral

side of the horizontal plane so as to form a diverticulum or blind sac above it; (5) the vagina which has well developed muscular layers and a mucosa which is devoid of the tubular glands characteristic of the rest of the oviduct. This gives it a different appearance from the uterine mucosa. The vagina is not straight but undergoes an 'S' shaped flexion on leaving the uterus. This bend in maintained in position by the muscles of the ventral ligament which at this point form a thick bundle known as the utero-vaginal muscles.

The cloaca is not a part of the oviduct. It is a vestibule into which opens the rectum and the ureters as well as the oviduct. The opening of the cloaca to the exterior, commonly known as the vent, is surrounded by a sphincter muscle. The cloaca also forms the insertion for the striated levator, retractor and dilator ani muscles which have their origin on the vertebral skeleton.

Physiology. The hen lays its eggs in clutches, that is a group of eggs laid on consecutive days each group being separated by a period of a day or more on which no egg is laid. A clutch may be from one to over a hundred eggs but an average bird will lay clutches of from two to six eggs with one or two days pause in between. Typically, the eggs of a clutch are laid progressively later in the day, the final egg being laid in the late afternoons. It is very unusual for eggs to be laid in the dark.

After ovulation, the ovum enters the infundibulum which at this time is very active. It takes about 15 minutes for the ovum to pass through the latter and about 3 hours for it to pass through the magnum where it receives its covering of thick albumen. An hour or more is spent in the isthmus

where the shell membranes are added. In the uterus, the membranous egg swells to nearly its final volume by the imbibition of water by osmosis through the membranes. This swelling or "plumping" occupies the first 8 hours of the 22 to 26 that the egg spends in the uterus. Shell secretion proceeds slowly until plumping is completed and then increases rapidly only to slow down again for the last 2 or 3 hours. It is not clear whether or not shell secretion ever completely stops; the work of Burmester, Scott & Card (1939) suggests that it does not.

Oviposition occurs 24 to 30 hours after ovulation and is followed within an hour by the ovulation of the next ovum. If the egg that was laid was the terminal egg of the clutch then the next ovulation does not occur until about 30 hours before the first egg of the next clutch is laid. Thus, if there is a day's pause between clutches then the first ovulation occurs some 12 to 15 hours after the oviposition of the terminal egg (Warren & Scott, 1935; Scott & Warren, 1936).

The release of "ovulating hormone" from the pituitary gland precedes ovulation by 4 to 6 hours (Rothchild & Fraps, 1949). Before this time hypophysectomy prevents ovulation, afterwards the capacity to ovulate resides within the follicle and will occur <u>in vitro</u> (Neher, Olsen & Fraps, 1950).

OVIPOSITION IN THE HEN

It is the purpose of this section to review in detail the physiology of oviposition starting with the egg lying in the uterus some hours before it is due to be laid. Oviposition consists of (1) the subsequent movements of the egg (2) the factors responsible for the initiation and control of those

movements; oviposition is accomplished when the egg finally lies outside the hen's body.

(1) THE MOVEMENTS OF THE EGG

The egg in the uterus

When the egg enters the uterus it has acquired its shape but not its maximum volume. The uterus, therefore, is distended by the entry of the membranous egg; it must distend still more during plumping and again, though negligibly, during shell formation. The increase in volume during plumping has been calculated by Bradfield (1951) by measurements from radiographs. He finds the initial volume to be 45 ml. which increases over the next 8 hours to nearly 60 ml. Therefore if the capacity of the empty uterus, as estimated from its weight, is 20 ml. then it must increase twofold when the membranous egg enters and three-fold by the time plumping has finished.

These details of egg volume are given because it is conceivable that distension of the uterus has some physiological significance either by its effect on the contractility of the uterine muscle (c.f. Starling's law of the heart) or by the stimulation of possible reflex arcs with motor nerves or hormones on the efferent side.

During its stay in the uterus the egg makes some small movements which coincide with respiration (Bradfield, 1951) and the presence of spiral markings on some shells suggests that rolling is possible.

While lying in the uterus the egg has the same orientation as it had when passing down the rest of the oviduct, namely with its pointed end nearer the vent (many references in Olsen & Byerly, 1932).

There is no well defined cervix in the hen but the sphincter muscle together with the convoluted

vagina are apparently sufficient to prevent the egg from inadvertently leaving the uterus. That the vaginal shape is effective in retaining the egg is suggested by the observations of Greenwood and Blyth (1938). These authors noticed that the vagina of certain experimental birds (as embryos they had been injected with oestrone) lacked the 'S' bend thus making it a straight tube. These birds had laid only soft shelled eggs (how many, and for how long is not stated) and it was suggested that this was because the eggs were not being retained long enough for shell secretion to occur. If so, then the soft shelled eggs would have been laid prematurely, probably within about five hours of their ovulation and it is unfortunate that the time of laying was not given.

The presence of the 'S' bend makes it very difficult to make a digital examination of the uterus per vaginam which suggests that a similar difficulty may be met by an egg travelling the other way.

It is well known that eggs can travel back up the oviduct from the uterus to the body cavity. Sometimes hard shelled eggs are found in the body cavity and various types of double eggs suggest that reverse peristalsis is possible. If the egg can move backwards, why does it not do so more often? There is no evidence of an occluding mechanism at the isthmo-uterine junction and therefore there would appear to be less resistance to the egg's movement in this direction. Since, however, the majority of eggs are probably not reversed, there must be a "physiological sphincter" at the isthmial end. Perhaps the tone of the isthmial muscles is great enough, under ordinary

circumstances, to prevent the uterine egg from reversing and, during oviposition, there may be active relaxation of the uterine sphincter.

Nesting behaviour

Strictly speaking this is a part of oviposition as defined above since it consists of the movement of the hen, still carrying an egg in the uterus, from the floor of the pen to the nest. Very little is known about this habit beyond the fact that it exists. The stimulus probably does not arise from the oviduct since regular nesting can be observed in birds which, for various reasons, are internal layers (Cole & Hutt, 1953) or in birds which have had their oviduct removed (see Appendix A).

Another important point is that the instinct is manifested not just before an egg is laid but from one to three hours previously with a mean time of 90 minutes (Turpin, 1918; Wood-Gush, personal communication).

It would be interesting to learn whether there is any connection between nesting behaviour and oviposition other than that both require a functional ovary.

Laying

(a) The orientation of the egg. The question as to which end of the egg is laid first has appeared many times in the literature. Since the egg may be laid either blunt end or sharp end first, this question does not appear to be one of major importance but, for completeness, a summary will be given.

It is clear that the egg passes down the magnum and comes to lie in the uterus with its sharp end leading. It is also well known that some eggs are laid blunt end first (Olsen & Byerley, 1932, put the number at 25%) hence between entering the uterus and

leaving the hen the egg must turn through 180°. Wickmann (1896) explained this turning on the grounds that the egg would sometimes get its sharp end so buried in the uterine blind sac that when the uterus contracted, the push from behind would turn the egg so that it was expelled blunt end first having turned 180° in the longitudinal plane. Curtis' (1916) observations that this sometimes happened on manually expelling an egg from a dead bird supported Wickmann's suggestion.

In 1951, Bradfield published a most interesting series of radiographs which showed the egg in the uterus before and after turning. In his paper he advanced two claims which contradicted earlier views. He deduced from these radiographs firstly that the egg turned horizontally through 180° and secondly that this turning occurred about an hour before oviposition and that it was the normal behaviour of the egg thus inferring that the majority of eggs are laid blunt end first.

The second claim appears to contradict the observations of Olsen & Byerly but the conditions of the two experiments were not similar.

(b) The passage of the egg out of the bird. Since the hen possesses a well developed vagina it would be natural to conclude that the egg passes through it during laying like a foetus passes through the mammalian vagina during parturition. This view was obviously held by Surface (1912), Curtis (1916), Greenwood (1935) and Jull (1952), but not by Wickmann (1896) who suggested that during oviposition the uterus, still containing the egg, was everted through the vagina in order to bring the egg to the vent. The uterus was then retracted leaving the egg outside

the hen having touched neither the vagina nor the cloaca.

Wickmann's suggestion has been accepted by others (Burmester, 1948; Romanoff & Romanoff, 1949) including Bradfield (1951) who observed oviposition on the radiographic screen and stated that uterine eversion did occur. It may be objected that it would be difficult to distinguish uterus from vagina radiographically and in the published photographs the oviduct is transparent to the X-rays.

There appears to be no other work on the movements of the egg, or the muscles involved during oviposition nor has any reference been found to any concomitant physiological effects on the blood pressure, body temperature or other organs and systems.

(2) FACTORS CONTROLLING OVIPOSITION

The ovary. It seems more than a coincidence that ovulation always occurs just after oviposition and never just before it (Warren & Scott, 1935) but though there are some suggestions that there may be a co-ordinating mechanism there is nothing to indicate how such a co-ordination works. Obviously the stimulus of an ovulation is not necessary for oviposition or the last egg of a clutch and the last egg of the season would never be laid nor is the converse true or a hen would never lay its first egg.

The effect of experimentally altering the times of ovulation and oviposition are summarized as follows:

Ovulation premature, oviposition premature.

Ovulation delayed, oviposition delayed (?)

Oviposition premature, ovulation not affected

Oviposition delayed, ovulation not affected

Premature oviposition was obtained by manually

expelling the egg (Warren & Scott, 1935) or by drugs (see page 14); delayed oviposition was obtained by drugs (see page 15). The fact that ovulation was not affected is probably because the capacity to ovulate resided in the follicle when the injections were made and the hastening or delaying agents acted directly on the uterine muscles.

More interesting is the effect of premature ovulation (Fraps, 1942) which was obtained by injecting luteinizing hormone while the terminal egg of a clutch was still in the uterus. This induced the ovulation of the first ovum of the following clutch more than half a day prematurely and also caused premature oviposition of the terminal egg. Not all the injections gave this result but it was found that if laying was not premature, then ovulation had not taken place. This work was confirmed by Nalbandov & Card (1946) who took the precaution of testing their luteinizing preparation for contamination by hormones of the neurohypophysis (q.v.) and found it negative.

Further evidence that the ovary exerts some influence on laying, comes from the work of Rothchild & Fraps (1944 a). They found that removal of post-ovulatory follicle (P.O.F.), the avian homologue of the corpus luteum delayed the laying of the egg which had originated in that follicle by as much as 9 days.

It is well known in mammals and in some viviparous reptiles that the corpus luteum exercises temporary or complete control over the fate of the uterine contents, and its removal leads to abortion and the termination of pregnancy. This function of the corpus luteum is attributable to the progesterone which it secretes.

This concept of the corpus luteum does not apply to the fowl for removal of the P.O.F. delays, not precipitates, laying and, moreover, although lutein cells have been described (Pearl & Boring, 1918), progesterone has not been detected by the McGinty test which is sensitive enough to detect the progesterone in corpora lutea (Riddle & Schooley, 1944).

It is interesting to note that Fraps, Hooker & Forbes (1948) have detected a progesterone-like substance in the blood of a laying hen by the very sensitive Hooker-Forbes test but this substance was also found in the blood of cocks (but not capons) and non-laying hens (Fraps, Hooker, & Forbes, 1949).

A point that was not mentioned by Rothchild and Fraps was whether ovulation continued after removal of the P.O.F.; if ovulation was interrupted, then the ovarian secretion of sex hormones would have been lowered. This could conceivably alter the properties of the uterine muscles although the authors showed that delayed eggs could still be expelled by the injection of oxytocics.

The oviduct. Rothchild & Fraps (1946), injected an oxytocic substance at different times after the egg entered the uterus. They found that the dose required to expel the egg decreased in proportion to the egg's volume and reached a minimum by the time that it was fully plumped. Their explanation that this was due to the increased contractility of stretched muscle is in keeping with what is known about the mechanical properties of muscle.

Hypophysectomy. Rothchild (1946) reports that oviposition was delayed in half of his hypophysectomized birds. He gives no figures for the length of the delay and, from an earlier paper describing the

operative methods (Hill & Parkes, 1934) it is clear that only the adenohypophysis was removed (the avian neurohypophysis is a descrete lobe enclosed in its own fibrous capsule). It remains to be seen whether the adenohypophysis plays a direct part in oviposition, perhaps through the ovary or adrenal glands, or an indirect part by its effects on the general metabolism.

Pituitrin. Premature laying was induced by the injection of mammalian pituitrin in the pigeon (Riddle, 1921) and later in the fowl (Burrows & Byerly, 1942). Burrows & Fraps (1942) extended this work on the fowl by showing that vasopressin was more efficacious than oxytocin and that the action of oxytocin could be accounted for mainly, but not completely, by its content of vasopressin. Morash & Gibbs (1929) showed that pituitrin would contract the oviduct *in vivo* (which part was not stated), and McKenney, Essex & Mann (1932) showed that it contracts the uterus *in vitro*.

Few people have worked with avian pituitrin. That such a substance exists was shown by Herring (1913) and Hogben & de Beer (1925) who prepared an extract of the neurohypophysis which had characteristic actions on blood pressure and the isolated mammalian uterus. It is not unlikely that avian pituitrin and its fractions would behave differently, quantitatively if not qualitatively, when tested on birds. Some experiments by the author have shown that pituitrin made from the glands of immature hens is at least ten times more potent in inducing oviposition than the commercial, mammalian hormone (see appendix B).

Other hormones. McKenney et al. (1932) have demonstrated the uterus-contracting action of acetyl

choline and histamine and the inhibitory action of adrenaline. Weiss & Sturkie (1952) found that oviposition was hastened by the injection of large doses of acetyl choline and histamine and was delayed by injections of ephedrine. The action of the first two drugs is readily understandable on the basis of their being uterine stimulants but the action of ephedrine presents some difficulties which will be discussed later (see page 75).

Sex hormones. Injections of certain sex hormones affect the time of oviposition. Progesterone and androsterone (1-10 mgm.) delay laying and ovulation in pigeons and hens (Dunham & Riddle, 1942; Neher & Fraps, 1950). Oestrogens may either advance laying (25% of the birds) or retard laying (50% of the birds). They can also retard ovulation for 24 hours without causing the atresia which often follows progesterone treatment (Dunham & Riddle, 1942).

Light. It has been stated that laying in the dark is very unusual. The reason for this, in the first place, is because ovulation is so timed that egg formation is never complete until the hours of daylight. It is not that the laying mechanism is inhibited by darkness for if ovulations are induced at the appropriate time the resulting eggs are laid in the dark (Neher & Fraps, 1950), and when the daylight is restricted to only 6 hours the first eggs of a clutch are laid in the dark (Byerly & Moore, 1941). Nor is there an endogenous diurnal rhythm since, under conditions of continuous illumination, eggs may be laid throughout the 24 hours of the day (Warren & Scott, 1936; McNally, 1947; Fraps, Neher & Rothchild, 1947).

There is one experiment on record which has been interpreted as meaning that light has a direct effect

on the time of oviposition. Rothchild and Fraps (1944 b) removed the post-ovulatory follicle, thus delaying oviposition, and then altered the hours of light and dark so that they were the reverse of the solar day; both reversed and normal days had 14 hours of light. They found that the delayed eggs were nearly all laid in the hours of light regardless of whether that coincided with the solar day or night. The control birds and the experimental birds which did not show a delay all laid at the expected time whether it was in the dark or in the light. Any influence of impending ovulations was excluded in this experiment by removing all the large follicles at the same time as the post-ovulatory follicle and all the eggs were laid within 3 days although it was a week before the next ovulation occurred.

It remains to be seen whether this interesting phenomenon plays any part in normal laying; it would be worth while repeating the experiment using other methods, for instance the injection of drugs or steroids, to retain the egg.

Fright. Several authors have observed that a frightening disturbance will often delay oviposition for up to 2 days and sometimes prevent ovulation as well (Patterson, 1920; Stieve, 1918; Warren & Scott, 1935; Scott, 1940; Sturkie, 1946).

Soft shelled eggs are sometimes attributed to the effects of fright, but they also occur in other circumstances e.g. after feeding sulphanilamide (Scott, Jungherr, & Matterson, 1944), after hypothermia (Sturkie, 1946), during a respiratory disease (Taylor, Gunns, Grau, & Lepkovsky, 1953), after the external application of nicotine (McKenney et al., 1932), and after surgical operations (Rothchild &

Fraps, 1945, 1947). Soft shelled eggs may be caused by a disturbance of either the secretory of motor functions of the uterus and it is conceivable that sometimes both functions may be upset. Since the above authors do not indicate whether the soft shelled eggs were premature or not it is impossible to speculate on the underlying causes. An interesting cause of soft shelled eggs is that of an irritant (a loop of suture silk) in the oviduct. This is explained more fully in appendix C.

CONCLUSIONS

The most striking impression given by this review is that much has still to be learned about oviposition.

The actual muscular contractions which constitute oviposition have not been described at all, neither is there a satisfactory description of the anatomy of the oviduct. Microscopically there are the "circular" and "longitudinal" muscle layers but it is unlikely that these layers exist simply as the hoops and slats of a barrel. They are more likely to form muscular bundles as in the human uterus which run obliquely in several directions. Certainly the "longitudinal" muscles at the caudal end of the hen's uterus seem to run almost circularly.

There is the question of the nature of the sphincter muscle, whether it has any characteristic properties; the nature of the vaginal muscles and the function of the peculiar utero-vaginal muscle. Wickmann's eversion theory has not been rejected nor confirmed.

Perhaps the most important unsolved problem is that of the nature of the stimulus which initiates uterine activity at the commencement of oviposition.

Three types of stimulus may promote the activity of smooth muscle:- nerve impulses which probably liberate adrenaline or acetylcholine-like substances e.g. control of the gut by the vagus nerve; hormones, which act directly on the smooth muscle e.g. pituitrin on the uterus; the muscle cells may show a spontaneous rhythm e.g. isolated circular muscle from the gut. Thus the hen's uterus may be stimulated to contract at oviposition by one or more of these agents but in the case of spontaneous contraction it is felt that there must be an inhibitory factor of some sort or one can see no reason why laying should not occur any time after plumping. An answer to this question of what is the primary motor stimulus that initiates oviposition would open the way to solving the problem of its control by the post-ovulatory follicle or the hypophysis.

In parenthesis it is interesting to note how the early success in the use of gonadotrophins in the induction of ovulation led to the present state of detailed understanding of this problem both physiologically and anatomically in many vertebrate species. By contrast our knowledge of birth processes has advanced very little since the nineteenth century.

It may be helpful to summarize the factors known to influence the time of oviposition:

Premature oviposition	Delayed oviposition
Induced ovulation	Removal of the P.O.F.
Pituitrin	Adenohypophysectomy
Acetylcholine	Ephedrine
Histamine	Progesterone
Surgical operations	Androsterone
Oestradiol	Oestradiol
	Fright

Only in the case of pituitrin and histamine is there an understandable mode of action because these

two substances act on the uterine muscle directly; so does acetylcholine but it also affects the central nervous system and other organs of the body thus making the simpler explanation unfounded but not unlikely. Ephedrine has many actions on the body similar to those of adrenaline, but it behaves differently in some respects and a fuller discussion of its possible mode of action will be given later (see page 75).

As for the other factors, their connection with oviposition is not at all obvious. Hypophysectomy, removal of the P.O.F. and induced ovulation might all be expected to interfere with the ovarian secretion of steroids and an approach along these lines, bearing in mind the importance of the time of injection relative to the laying cycle, would, perhaps, be profitable. It must also be remembered that any effects of steroid hormone imbalance must be rapid ones because, in the normal course of events, the sequence of ovulation - "gestation" - oviposition occurs every 24 hours.

AN OUTLINE OF THE EXPERIMENTS

The object of the experiments was to investigate the mechanics of oviposition and to ascertain the method by which the egg was ejected from the uterus. Confirmation of Wickmann's theory of uterine eversion was sought and the motility of the uterus and vagina was to be investigated. In accomplishing this aim, several incidental observations were made which led to further experiments on other aspects of oviposition.

The results are presented in three sections:

(1) The motility of the uterus and vagina in vitro. The classical Magnus method was used as a convenient way of demonstrating whether any differences in activity and reactivity exist between the uterus and vagina and between different regions within each of these parts.

It might be expected, if Wickmann's theory were true, that the vagina would augment the uterine contractions. Also, it is known that in the rabbit, the uterus at the time of parturition does not show uniform motility but is differentiated in such a way that the fundal contractions are dominant (Bonnycastle & Ferguson, 1941).

(2) The effect of adrenaline on oviposition. These experiments followed from the previous section in that the results of adrenaline on the uterus in vitro were extended to the living, intact bird in order to assess their functional significance.

(3) The role of the abdominal muscles in oviposition. In this section the effects of laparotomy and spinal cord transections on oviposition are recorded. Certain observations that were made on the

"oviposition-efforts" induced by stimulation of the vagina, led to the discovery of a reflex by which the abdominal muscles assist in oviposition. Several features of this reflex were examined including its functional significance.

In the Appendix, which precedes the discussion, several minor experiments are described the results of which are only tentative or which deal with other aspects of oviposition.

METHODS

Birds. Throughout these experiments Brown Leghorn hens of various ages were used. They came from several of the inbred lines and from crosses between those lines, which are kept at the Poultry Research Centre.

The birds were housed either in indoor pens fitted with trap nests or in individual cages; egg recording was carried on continuously.

Though the Brown Leghorn is an excitable bird, it responds to training and there was usually no trouble in handling the birds for palpation of the oviduct or injections. This was particularly true of those kept in cages.

Palpation of the oviduct per rectum is a simple way of deciding whether a bird may be expected to lay on a particular day. The hard shelled egg is easy to feel and with a little experience a soft shelled egg may be felt on the day before it is laid. This is very useful as it enables one to predict the start of a new clutch which would commence in the early morning.

The motility of the oviduct in vitro. The contractions of isolated strips of muscle were recorded by isotonic levers. The arrangement of 2 inner vessels in the water bath enabled 2 preparations to be running at the same time, each with a separate inflow and outflow system. By means of an overflow tube connected with a water pump the volume of perfusing fluid in the vessels was automatically regulated.

Two strips of muscle about 3 x 1 cm. were cut from the uterus or vagina in a longitudinal or circular direction (circular here meaning a transverse section since strips not loops were used). These

were suspended in the inner vessel for about half an hour until a constant pattern of motility was established. The drugs were added at sub-threshold doses and subsequently increased in amount. After a response the preparations were washed by overflow, the original levels restored and the next experiment started when the preparations were normally active.

Perfusate. Ringer-Locke, Dales and Tyrode solutions were all tried but without any difference being seen. Ringer-Locke was generally used. Its tonicity was compared with that of various salt solutions by their effect on erythrocytes. There was a fairly large range (0.75% - 1.1%) over which neither haemolysis nor crenation occurred and Ringer-Locke fell well within this range.

Contrary to the experience of McKenney et al., it was not found necessary to use only glass distilled water.

Temperature. The water bath was kept at about 42°C. with a variation of not more than 0.5°F either way. McKenney et al., perhaps forgetting that the fowl has a higher body temperature than mammals, kept their preparation at 39°C.

Oxygenation. Pure oxygen was used mostly, after it was shown that a mixture of 95% oxygen and 5% carbon dioxide was not obviously any more suitable.

Storage. McKenney et al. found that the motility of the oviduct was almost completely lost after storage in the refrigerator overnight. In the present work this was not found to be the case; overnight storage at from 1 - 3°F enabled strips of muscle to be used the next day without impairing their spontaneous motility or their reactions to drugs.

There was a reduction in their activity on the third day but even by the fourth day some activity persisted.

Drugs. Racemic adrenaline hydrochloride (B.D.H.), ephedrine sulphate (Boots) and Pituitrin (Parke Davis) were the principle drugs that were used. At low concentrations the adrenaline was prevented from oxydozing by the addition of ascorbic acid (50 mg.%). The volume of the inner vessel was 17 ml. The concentrations of the drugs are recorded as the final concentrations achieved in the inner vessel but, for brevity, instead of writing 1 μg in 17 ml. as 1:1.7 x 10^7 only "10^7" will be written. For pituitrin the same system is used but the figures refer to International Units and not to grams. The tracings are always typical of the results which were obtained in at least two preparations per bird and in several birds. Altogether 18 birds were used for this work.

Anaesthesia. For the smooth induction of anaesthesia, free from any struggling, intravenous veterinary "Nembutal" was used (10% Na pentobarbital, Abbott). This was injected slowly until the bird no longer withdrew its head upon pinching the comb. At this stage, after about 0.75 ml. had been injected, the bird would be anaesthetized for about 15 minutes. For longer anaesthesia sometimes more Nembutal would be given intra-peritoneally but usually ether was used as it was readily controlled. Profound anaesthesia for several hours duration was obtained by intra-peritoneal phenobarbitone at a dose of 200mg./kg. For local anaesthesia 2% Nupercaine or 10% Procaine was injected into the tissues.

Spinal cord transection. The spinous process of the vertebra was removed by bone forceps after separating it from the surrounding vertebral muscle. The spinal cord was exposed by drilling through the body of the vertebra and then removed in pieces with fine forceps. A probe was passed down the remaining spinal cord below the drill hole to completely disorganize the cord for 1-2 cm. below this point. The control operation consisted of removing the vertebral spine and drilling the body for a little way. The lesions were made at segments higher than L6 below which arise the autonomic nerves to the reproductive organs.

The spinal birds all suffered paralysis of the abdomen and hind limbs and had to be hand fed because of their inability to move to the feeding troughs. All of them showed a complete loss of the tail stabilizing reflex when they were tilted in the vertical plane and an insensitivity to painful stimuli caudal to the lesion, but some of them continued to withdraw the legs from a painful stimulus.

Respiration recording. The recorder designed by Gaddum (1941) was tried but it did not work satisfactorily owing to its large resistance and finally a T tube inserted in the trachea was used, the fall in pressure in one arm being recorded by means of a tambour. This device recorded the frequency and relative changes in the depth of the respiration.

Intra-abdominal pressure. A small balloon 3 cm. long was made from a finger cot. It was inserted through an incision in the abdominal wall which was closed before dilating the balloon with warm saline. The balloon was connected with a mercury manometer recording on a kymograph.

Distension of the vagina and rectum. A balloon made from a finger cot was attached to a Marriotte bottle filled with warm saline. The pressure in this reservoir could be raised by means of a hand bellows and then lowered by opening a spring clip. Thus the balloon could be dilated to any size but usually it did not exceed the volume of an egg, 40-60 ml. The vagina and rectum were easily entered once the cloacal lips were held apart by forceps.

RESULTS

1. THE MOTILITY OF THE UTERUS AND VAGINA

The uterus

There was a certain amount of variability in the pattern of motility shown by the uterine strips but nearly all the birds were killed at about the same time in the laying cycle so as to minimize any effects that this factor may exert. The birds were usually carrying a hard shelled egg so that initially the degree of uterine distension would be similar in each.

In any one bird the motility of the circular and longitudinal muscles was the same and no differences could be found between different regions of the uterus.

The uterus of a non-laying hen showed contractions of increased frequency but of considerably reduced amplitude when compared with a laying hen (fig. 1). McKenney et al. found no spontaneous activity in the non-laying hen's oviduct but it is possible that this was a result of the lower temperature of the water bath.

Adrenaline. McKenney et al. have shown that adrenaline inhibits the motility of the hen's uterus and this was confirmed by the present experiments. In addition it was shown that this response was obtained from circular and longitudinal muscles in any part of the uterus (fig. 2). The lowest effective concentration was 10^{8} but the effect was of less than a minute's duration. A high concentration (10^{5}) would cause a prolonged inhibition lasting for over 30 minutes.

While it is inhibited by adrenaline the uterus is insensitive to pituitrin at concentrations which are normally effective in stimulating activity (10^{3}; fig9b).

At higher concentrations of pituitrin (10) there is a weak response (fig. 3). Conversely, a large dose of pituitrin (10^4) will prevent adrenaline from exerting its inhibitory effect even when present in concentrations of 100 x threshold (fig. 3).

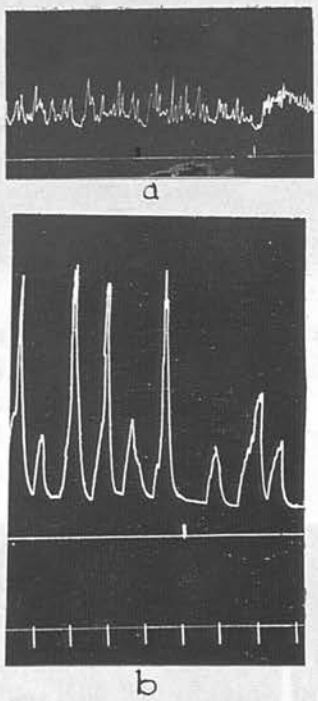


Fig. 1. Uterus; circular muscle
(a) Non-laying hen,
(b) Laying hen.
Adrenaline (10^7) added at the mark.
Time scale, in minutes, the same for figs. 1-12.

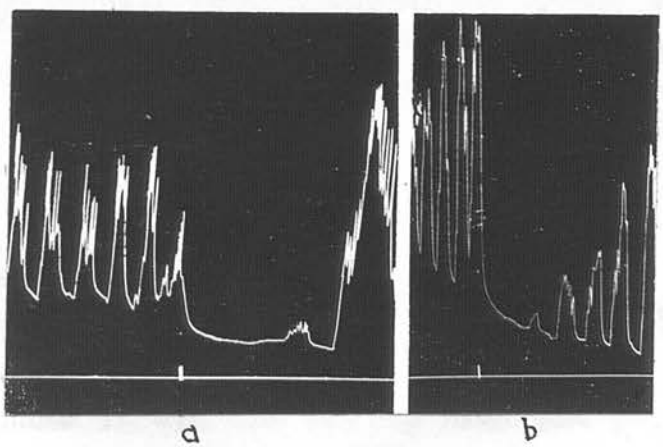
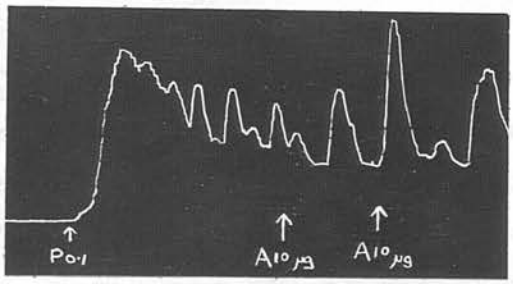
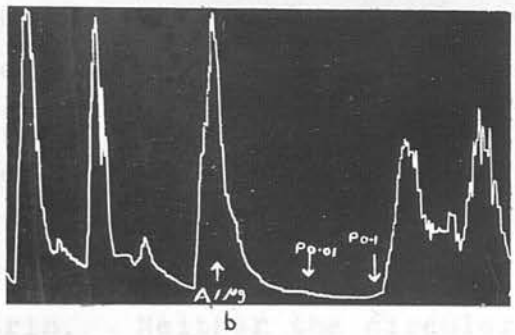


Fig. 2. Uterus; (a) circular muscle
(b) longitudinal muscle.
Adrenaline (10^7) added at the mark.



a



b

Fig. 3. Uterus; longitudinal muscle
(a) pituitrin (10^2) followed by adrenaline 10^6;
(b) adrenaline (10^7) followed by pituitrin 10^3, 10^2.

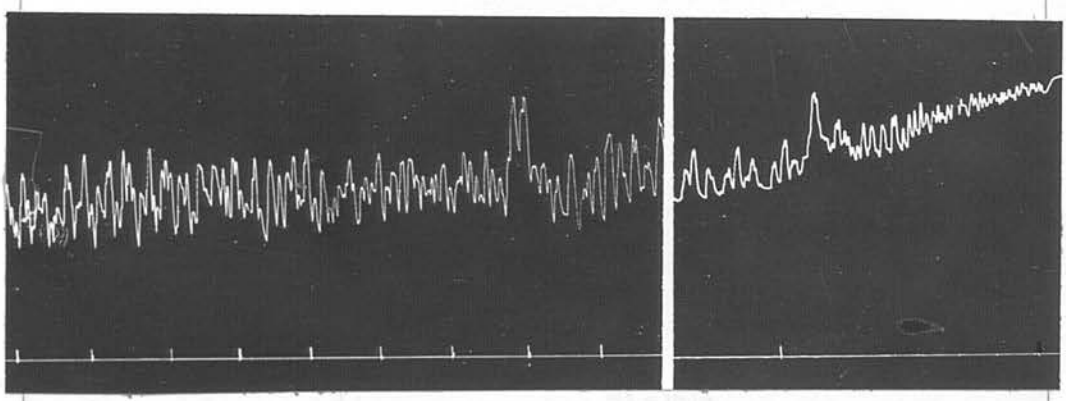


Fig. 4. Uterus; longitudinal muscle.
From left to right, the marks indicate the following concentrations of ephedrine: 10^{10}, 10^9, 10^8, 10^7, 10^6 10^5, 10^4, 10^3, 3×10^3, 5×10^3.

The vagina

The spontaneous contractions were always small in amplitude and often fairly quick. The response was the same in all the laying hens but the vagina of the non-laying hens was almost completely motionless and unreactive to drugs (fig. 9c).

Adrenaline. The circular muscle always contracted quickly in the presence of adrenaline; 10^8 was threshold for this response (fig. 11a). The contraction was usually tetanic and relaxation was procured by washing the preparation.

The longitudinal muscle, on the contrary, was unresponsive to adrenaline even in concentrations as high as 10^5 (fig. 11b). Occasionally a large dose of adrenaline would slowly increase the tone but never in the rapid way that it produced tetanus of the circular muscle.

Pituitrin. Neither the circular nor the longitudinal muscles showed any response to concentrations of pituitrin which were effective on the uterus (10^3-10^2). A higher concentration (10) sometimes raised the tone of a preparation but equally often was without effect (fig. 9 & 10). This amount of pituitrin is as much as is required to produce premature oviposition in the intact bird.

The differences in reactivity between the uterus and the vagina may be summarized in the following table which gives the type of response and threshold concentrations of adrenaline and pituitrin

	Uterus	
	Circular	Long
Adrenaline	Inhibits (10^8)	Inhibits (10^8)
Pituitrin	Motor (10^3)	Motor (10^3)

	Vagina	
	Circular	Long
Adrenaline	Motor (10^8)	No action (10^5)
Pituitrin	Tonic (10)	Tonic (10)

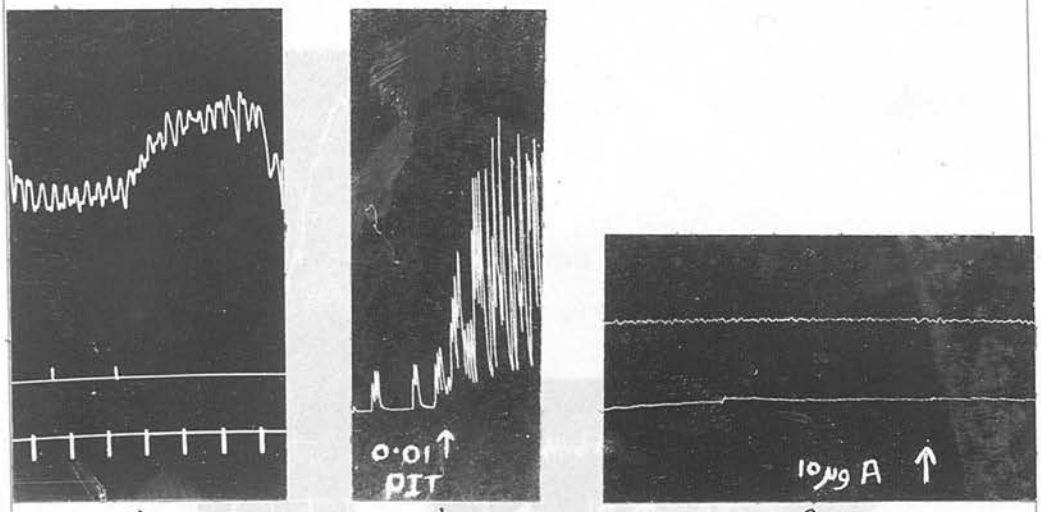


Fig. 9: Vagina (a): circular muscle, effect of pituitrin (10^2, 10), time in minutes; (b) uterus, longitudinal muscle showing effect of pituitrin (10^2) for comparison; (c) vagina of non-laying hen: top: longitudinal muscle, below: circular muscle, adrenaline (10^6) at mark.

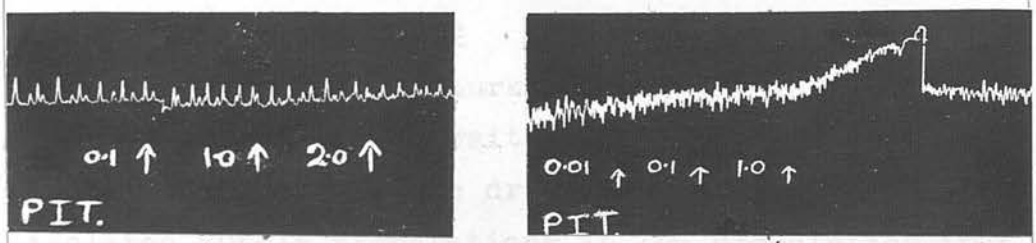


Fig. 10: Vagina; longitudinal muscle.
(a) Complete insensitivity to concentrations of pituitrin (10^2, 10, 2 x 10).
(b) Rise of tone following pituitrin (10).

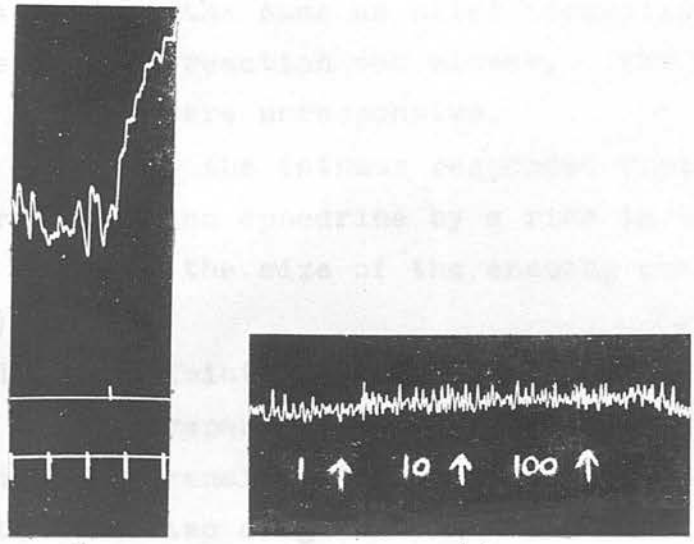


Fig. 11: Vagina.
(a) Circular muscle; effect of adrenaline (10^{7}).
(b) Longitudinal muscle; effect of adrenaline (10^{7}, 10^{6}, 10^{5}).

The action of Ephedrine

Since Weiss and Sturkie (1952) have shown that ephedrine delayed oviposition and since it is known to be a sympathominetic drug, it was tested on the isolated muscle preparations in the expectation that it would act like adrenaline.

Ephedrine did not inhibit the motility of either circular or longitudinal muscles of the uterus at concentrations up to 10^{3}. At higher concentrations (5×10^{3}) there was a steady rise of tone and diminution of amplitude which terminated in a state of tetany (fig. 4). Therefore the action of ephedrine was not similar to that of adrenaline on the uterus.

The vagina was more reactive to ephedrine (fig. 12). The circular muscles went into a state of tonic contraction at concentrations of 10^{5} to 10^{4}. This might be called a slow adrenaline response in that the

final result was the same as after adrenaline but the speed of the reaction was slower. The longitudinal muscles were unresponsive.

In contrast, the isthmus responded rapidly to both adrenaline and ephedrine by a rise in tone which did not suppress the size of the ensuing contractions. (fig. 5).

Gaddum and Kwiatkowski (1938) suggested that many of the discrepancies shown by ephedrine when compared with adrenaline may be explained on the theory that the two drugs interact in a way similar to that shown by eserine and acetylcholine. At low concentrations there is a synergistic action and at high concentrations they are antagonistic.

This type of action was sought on the hen's uterus but no synergism was found at any concentrations of ephedrine (fig. 6). There was, however, evidence of the antagonistic action. A concentration of ephedrine of 10^4, which by itself had no effect on the uterus, depressed the response to adrenaline so that the effective dose was raised from 10^8 to 10^5, and greater amounts of ephedrine abolished the response to adrenaline altogether (fig. 7).

When a large dose of ephedrine was used (5×10^3), the uterus could no longer respond to pituitrin (fig. 8a) but a moderate amount (10^4) did not have this effect. Moreover, when the uterus has been stimulated by pituitrin, even high concentrations of ephedrine were ineffective just as were high concentrations of adrenaline (fig. 8b).

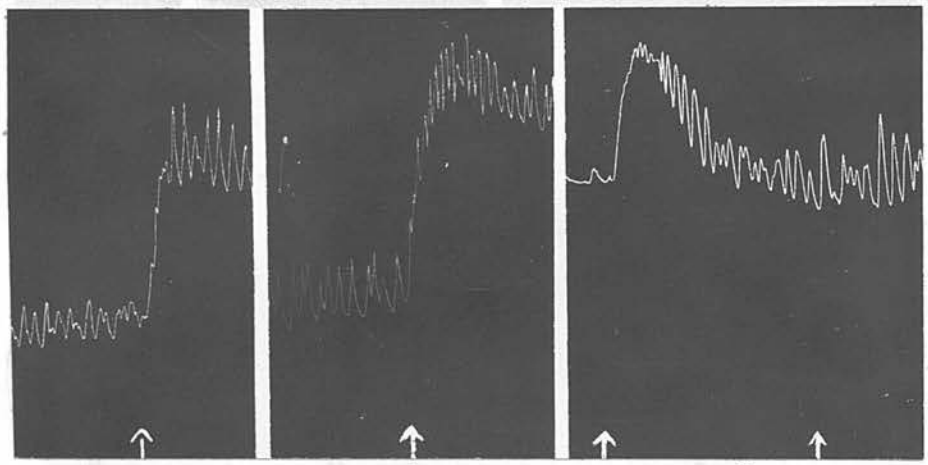


Fig. 5: Isthmus; circular muscle (a) effect of adrenaline (10^{8}); (b) effect of ephedrine (10^{4}); (c) effect of ephedrine (10^{4}) followed by adrenaline (10^{8}).

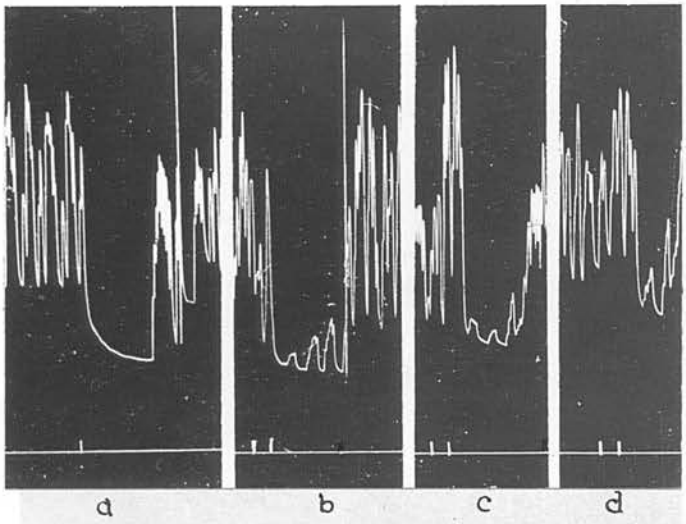


Fig. 6: Uterus; longitudinal muscle. (a) effect of adrenaline (10^{8}), (b)(c)(d), effect of ephedrine (10^{8}, 10^{9}, 10^{10}) first of each pair of marks followed by adrenaline (10^{8}). Note absence of synergism.

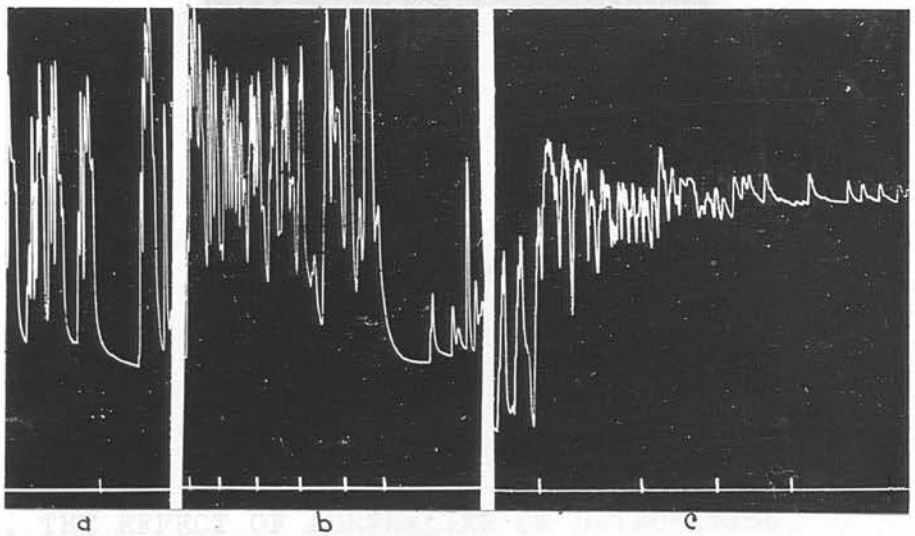


Fig. 7: Uterus; longitudinal muscle. (a) adrenaline (10^{8}); (b) ephedrine (10^{4}) followed by adrenaline (10^{8}, 10^{7}, 5×10^{7}); (c) ephedrine (5×10^{3}) followed by adrenaline (5×10^{7}, 10^{6}, 10^{5})

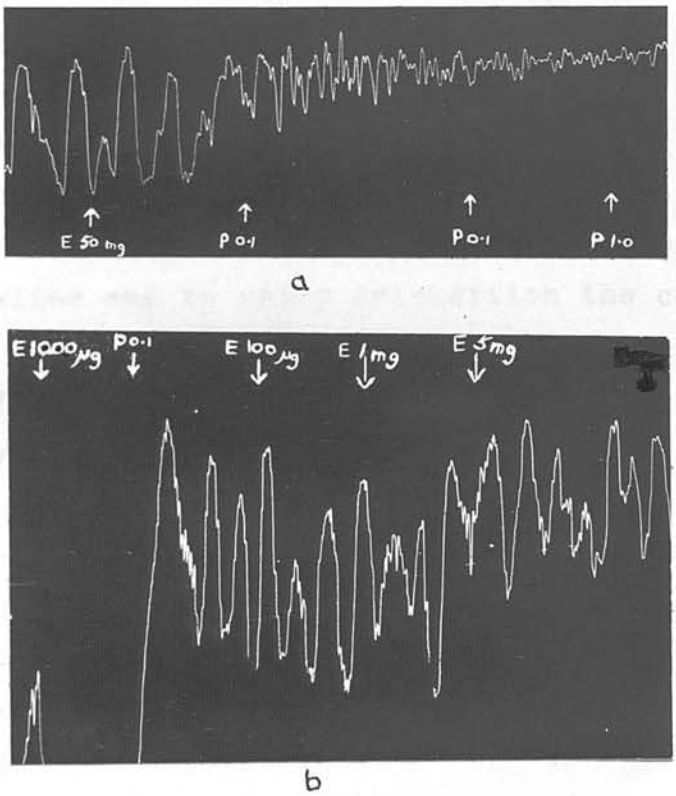


Fig. 8: Uterus; longitudinal muscle (a) ephedrine (5×10^{3}) followed by pituitrin (10^{2}); (b) ephedrine (10^{4}) followed by pituitrin (10^{2}) and ephedrine (10^{5}, 10^{4}, 5×10^{4}).

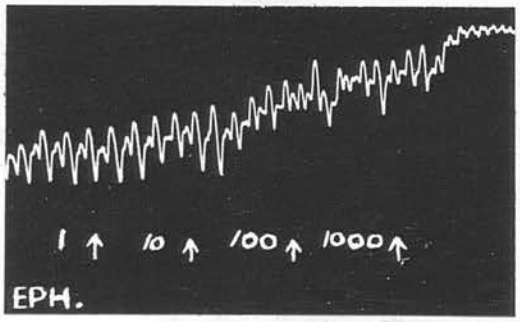


Fig. 12: Vagina; circular muscle. Effect of ephedrine (10^7, 10^6, 10^5, 10^4).

2. THE EFFECT OF ADRENALINE ON OVIPOSITION.

In the previous section it was shown that adrenaline strongly inhibited uterine movements and their response to pituitrin. It should follow then that, by its anti-expulsive action, adrenaline would disturb oviposition if injected at the appropriate time.

Doses of adrenaline starting from 0.25 mg. were injected subcutaneously at 10.00 hours if a hard shelled egg was present in the uterus. The eggs were mainly the first or second ones of a clutch; some were the third to the seventh egg. The effect of adrenaline was to delay oviposition the criterion of delay being the failure of oviposition to occur before 17.00 hours on the day of the treatment. Undoubtedly a few eggs are normally laid later than this but many eggs, particularly the first of the clutch, could be delayed for 6 hours and still be laid before 17.00 hours so the error is considered to under-estimate the number of delayed eggs.

The results are summarized in columns 1, 2 and 3 of Table 1. The majority of delayed eggs were laid by 09.00 hours the next day, but some were held for as long as 48 hours after the expected time.

Table 1. The effects of adrenaline on oviposition and ovulation

Dose of adrenaline (mg.)	0	0.25	0.5	1.0	2.0	5.0	10.0
No. of eggs *in utero* on day 1	20	3	3	22	47	1	2
No. of eggs delayed	0	0	1	20	44	1	2
% of eggs delayed	0	0	33	91	91	100	100
No. of eggs laid on day 2*	9	-	-	9	7	-	-
% of No. in utero on day 1	45	-	-	45	15	-	-
No. of days from day1 until laying of next egg. Mean±S.E.	2.0 ±0.3	- -	- -	2.8 ±0.6	4.0 ±0.3	- -	- -
Drop in No. of eggs laid in 10 days from day 2. Mean±S.E.	0.5 ±0.2	1.3 -	1.8 -	2.4 ±0.6	2.6 ±0.6	8.0 -	7.0 -
% Fall in egg production	7.3	20.6	23.1	35.7	42.1	100	95.9

* These eggs were not the same as those in row 1, but were the next in the clutch.

Adrenaline is widespread in its actions on the body but in view of the *in vitro* findings the most likely site of action in these experiments is the uterine muscle. If, so the antagonism between adrenaline and pituitrin, which has been seen *in vitro*, should also be seen when the two substances are injected *in vivo*.

Therefore the ability of pituitrin to induce oviposition was tested at various times for 2-240 minutes after the injection of 1 mg. adrenaline. From previous experience it was known that 1 unit of pituitrin intravenously would always induce oviposition within 5 minutes and the effective dose by the intra-muscular route was 5 units and the time taken was 15-20 minutes. Intra-muscular injections were often made when a

previous intravenous injection had made a large haematoma which obscured the course of the brachial vein.

Table 2. The ability of Pituitrin to induce oviposition after the injection of 1 mg. of adrenaline.

Mins. after injection of adrenaline.	Intravenous			Intramuscular			Total		
	Total	No. Laying	% Laying	Total	No. Laying	% Laying	Total	No. Laying	% Laying
2-4	6	5	83	-	-	-	5	5	83
5-10	10	5	50	7	1	14	17	6	55
15-30	2	0	0	5	0	0	7	0	0
60-90	4	2	50	5	0	0	9	2	22
120-150	8	4	50	8	0	0	16	4	25
180-210	1	1	100	9	4	44	10	5	50
> 240	19	17	89	9	2	22	28	19	68

The results are set out in Table 2. It is obvious that the characteristic action of pituitrin had been impaired; between 5 and 150 minutes after administering the adrenaline, never more than 50% of the injections successfully induced laying. It is unfortunate that only 2 experiments were made between 10 and 60 minutes after the adrenaline for it is impossible to say with so few results whether or not the uterus was completely refractory. Normal reactivity of the uterus to intravenous pituitrin was restored within 4 hours but the more slowly acting intra-muscular pituitrin was still not very efficacious at this time.

Besides delaying oviposition, adrenaline brought about a fall in egg production. Two aspects of this were considered. First, the effect of adrenaline on ovulation that was imminent at the time of injection.

If the hens had been very regular layers then it would have been a simple matter to predict the number of eggs which should have been laid by the group on the day after the adrenaline treatment and to compare this with the actual number of eggs that was laid. In this way, the treated birds would have served as their own controls. Since, however, the birds were frequent, but not regular, layers this could not be done with any accuracy and instead the number of eggs they laid was compared with the number laid by the control hens following a mock injection. Only the results from the 1 and 2 mg. groups gave sufficient data for this comparison; the figures are given in column 4 of Table 1.

It is evident that 2 mg. but not 1 mg. adrenaline often prevented the ovulation of the next egg. In both groups the next egg was often soft shelled and was laid early in the morning along with the delayed egg.

The second approach was to see if there was any effect on the number of ovulations over a certain period compared with the number in the same period before the injections, each hen thus being its own control. The mean fall in production and, where there were enough observations, the standard error, were calculated for each group and the significance was estimated by the 't' test.

It can be seen from Table 1, column 6 that with increasing doses of adrenaline the egg production dropped. Ovulation appeared to decline even in those groups in which the amount of adrenaline was too small to delay oviposition but since the numbers involved were small, this may have been caused by a

sampling error.

In column 5 of the same table, the number of consecutive "egg-free" days from the day after injection is given. This figure was calculated to determine whether the drop in egg production occurred immediately after the adrenaline treatment or whether it was spread out over the whole ten day period. The results suggested that a sharp fall occurred in the period immediately after injection when the larger dose was given.

The significance of the drop in egg production of the two groups when compared with the controls and with each other was:

	1 mg.v control	2 mg.v control	1 mg.v.2mg.
Egg production in 10 days	P = 0.01	P=0.001	Not Sig.
No. of days from injection to first egg	Not Sig.	P=0.01	Not Sig.

3. THE ROLE OF THE ABDOMINAL MUSCLES IN OVIPOSITION

Observations during laparotomy

Laparotomy was performed in 9 birds which were carrying a hard shelled egg in the uterus and which were expected to lay at about the time of the operation. The extent of the incision varied so that in some the whole of the uterus and vagina was visible while in others the position of the egg was felt by digital examination. Oviposition was initiated naturally during the operation in all the birds.

Laying was successfully accomplished in 6 of the hens but whereas oviposition in a supine, anaesthetized hen may take up to 5 minutes after the egg first appears at the vent in these hens it took an average

of 12 minutes before the egg was finally expelled. The times for the 6 birds were: 8, 19, 15, 15, 8 and 6 minutes.

The remaining 3 hens had not laid within 60 minutes although for all this time they had been making "bearing-down" efforts. Two units of pituitrin, intravenously, were injected into 2 of these birds but without success and the eggs were then removed by hand. The third bird had its abdomen closed and was returned to its cage where it laid about 3 hours later.

The explanation of this protracted labour would appear to be that the abdominal muscles normally aid in the expulsion of the egg and their muscular force is impaired when they are severed. The three birds which were unable to lay their eggs all had long incisions stretching from the cloaca to near the tip of the metasternum. Also, if the abdominal muscles merely contained an increased abdominal pressure rather than actively contracting themselves then a small incision should have been as effective in protracting or preventing oviposition as a large one.

If the damage to the abdominal muscles was not the cause of the derangement then the general effects of operative trauma (e.g. histamine releases as in mammals) may be implicated. The effect of posture may be ruled out since it could be shown that oviposition proceeded normally in 7 anaesthetized birds lying prone or supine.

In all the laparotomized birds the egg was delayed in the vagina and not in the uterus which was either seen or felt to be empty even though the egg had not been laid. This observation very strongly

suggests either that Wickmann's theory of uterine eversion is not correct or that oviposition in these birds was of an abnormal type. In 3 birds, the egg was observed to pass from the uterus to the vagina within the space of a minute.

There are some further observations which also are difficult to interpret in terms of Wickmann's theory.

(a) It was shown in Section 1 that the vagina and the uterus differed in their response to adrenaline and pituitrin. It is difficult to envisage what would follow if adrenaline were released while the uterus was everted into the vagina since the inner muscles of the uterus would be inhibited, and the outer ones of the vagina, would be contracted.

(b) If the tissue which surrounds the egg at the time of laying is held with forceps and examined it will be seen that the mucosa is vaginal and not uterine; the two types are readily distinguishable.

(c) Prolapsus, a common occurrence in hens, which Burmester (1948) attributes to "the failure of the uterus to be drawn back after oviposition", is mostly, in the author's experience, a prolapsus of the vagina. This condition may affect the uterus at times, as it does in mammals, but it has not been observed. Three birds suffering from a typical prolapsus of the vagina were examined by laparotomy and the uterus was seen to be in its normal position.

(d) Examination of the hen's uterus does not reveal any means whereby it could be everted. It is held in position by the dorsal ligament which, while it provides a little "slack", would hardly allow complete eversion.

The conclusions that may be drawn from these observations on laparotomized hens are (1) that the intact abdominal muscles are probably necessary if oviposition is to proceed at its normal rate and (2) that the egg passes from the uterus to the vagina before it is laid.

Observations on spinal hens

If, as suggested above, the abdominal muscles are normally used during oviposition then results similar to those obtained in the laparotomized birds might be obtained if the motor nerves to these muscles were interrupted by spinal cord transection. But this could happen only if cord transection did not interfere with the normal process of initiation of oviposition leading to the successful expulsion of the egg from the uterus. There were then two questions to be answered:- Could oviposition be initiated in the spinal hen and could it be successfully accomplished? Or, put another way, were the nerves passing from the higher centres of the uterus and to the vagina and abdominal muscles essential for oviposition?.

Nine spinal birds were prepared at different times each carrying a uterine egg at the time of the operation. It was hoped that ovulations would continue normally and that several eggs would be obtained from at least some of the birds but one of the results of cord transection was that ovulation was almost entirely inhibited during the time that the birds survived. In the 51 "hen-days" after the operation there were only 2 ovulations compared with 29 in the same period before. These 2 ovulations may have been impending at the time of the operation and were not

affected by the inhibiting agent.

On examination post-mortem there was evidence of follicular atresia in the ovaries of most of the birds (Table 3). Only two birds, one which had been treated with hormones and one which had died the day after the operation, were without atretic follicles. In a group of 18 normal hens, killed in laying condition, only 2 atretic follicles were found.

Table 3. The state of the ovary of spinal hens.

Hen No.	Survival (days)	No. of eggs in 30 days before Section	No. of Ovarian Follicles Weighing over 1 gm.	
			Normal	Atretic
H	(9)	20	8**	0
I	(6)	20	0	2
J	(6)	19	5	3
K	(16)	22	4	8
L	(5)	15	12	1
M	3	18	4	2
N	3	16	3	1
O	1	15	5	0
P	2	9	1	4
18 Normal hens	-	-	Mean 4.4	Mean 0.11

*Birds which were killed are in parentheses.
**Follicular growth stimulated by P.M.S.

In order to obtain more eggs from the spinal birds 2 of them (H and L) were injected with 800 I.U. pregnant mares' serum gonadotrophin (Organon Co.) intravenously. One of them (H) had been treated with the same amount of gonadotrophin subcutaneously 3 days previously to promote follicular growth. As a result of this treatment 2 further eggs were obtained making a total of 13 before the experiments

were discontinued owing to the poor condition of the birds.

Oviposition was considered to have been initiated normally if the egg left the uterus and was observed at the vent (the observations at laparotomy having shown that at this time the egg was in the vagina) or if it was successfully laid. The time when either of these events occurred is given in colum 6 of Table 4.

Table 4. The initiation of oviposition after spinal cord transection.

Hen No.	Position of egg in clutch	Time of operation	Lesion	Initiation	Approximate Time of Initiation	Remarks
H	3	9.30	L2-3	N	10.00	Soft-shelled, laid between 10 and 23 hours after operation
	4			N	-	
	Induced			O	-	P.M.S. day 4; in utero days 7.8.9. Induced by Pituitrin on day 10.
I	1	10.00	L8	N	10.30	
J	3	10.00	T6-7	N	16.00	
K	3	11.00	L1-2	N	15.30	
	1			N	9.00	On day 2
L	1	10.00	T6	N	10.30	
	Induced			N	-	P.M.S. day 1; in utero day 2; laid day 3.
M	2	17.00	T5	N	16.00	On day 1
N	2	10.00	L2-3	O	-	In utero day 2; induced by Pituitrin
O	1	17.00	T7	O	-	In utero day 1; died later
P	1	17.00	L2	O	-	In utero day 2; died later
Q	2	10.00	Control	N	13.00	
R	3	10.30	Control	N	16.45	
S	2	10.00	Control	N	16.00	

Day 1, 2, 3, etc. refer to the days after cord transection.
P.M.S. = pregnant mares serum gonadotrophin.
N = oviposition naturally initiated;
O = oviposition not naturally initiated.

Of the 13 eggs, 9 were initiated normally; of the 4 which remained in the uterus 2 were laid as a result of pituitrin treatment thus indicating that the uterine muscle was still reactive. The details of the birds are given in Table 4. The birds O and P

were carrying the first egg of the clutch at the time of the operation and should have laid within 12 to 18 hours so it was unlikely that they were prevented from laying by their early death which was not until between 26 and 36 hours later.

There were 11 eggs, from the spinal hens, which reached the vagina (9 by normal processes and 2 after pituitrin treatment) and of these 10 were successfully laid. The one bird which failed to lay (bird N) was in good condition on the second day after the operation and oviposition was initiated by 5 units of pituitrin intramuscularly. This brought the egg to the vagina where it remained for 10 hours and the bird then died with the egg unlaid. In some of the birds oviposition was observed and it was clear that the process took much longer than normal. Also, as far as could be judged, the eggs were laid without the usual straining efforts and the bird was oblivious to the fact that laying was in progress.

The table lists the 11 eggs in the experiment and the length of the delay that was observed. In 7 normal birds the mean time that the egg remained at the vent before it was laid was 1.8 minutes.

Egg No.	1	2	3*	4	5	6	7	8	9	10	11*
Hen No.	H	H	H	I	J	K	K	L	L	M	N
Days after operation	0	1	9	0	0	0	1	0	3	1	2
Time at vent (mins.)	75	-	15	30	-	10	-	165	-	-	(600)**

*Induced by pituitrin
**Remained in the vagina

The bearing-down reflex and oviposition

When the egg left the uterus and entered the vagina active movements were initiated which at once suggested that the bird was "trying" to lay its egg.

The abdomen contracted and the respirations increased, sometimes there were gasps indicating that a strong muscular effort was being made.

The cause of these bearing-down efforts was, possibly, the presence of the egg in the vagina which stimulated nerves ultimately connected with the abdominal and respiratory muscles. That such a reflex existed was suggested by some observations made on a bird which was being treated for a prolapsus. In such cases it is usual to clean the vent and replace the everted tissue which should then stay in position. If it does not then it may be necessary to insert a purse-string suture around the vent.

The vagina of the bird was pushed back into place but without success for it was promptly everted again. To overcome this the hen was lightly anaesthetized, in order to insert a purse-string suture, but when the vagina was pushed back into position again the hen immediately commenced to "lay" showing all the features mentioned above and finally everting the vagina. Whenever the vagina was stretched the strong expulsive efforts of the hen could be felt by the hand. Another feature in common with normal oviposition was the erection of the feathers over the lower half of the abdomen.

There was no evidence that a reflex of this nature had been described before in birds although the following quotation from Pearl, Surface & Curtis (1915) seems prophetic in this respect: "Zürn says that faeces may become lodged in the cloaca and then set up the same expulsive reflexes as an egg in the cloacal or vaginal regions does. In the effort to expel this foreign body the oviduct may become everted."

The following account of this phenomenon in the hen is divided into two sections, one describing the erection of the abdominal feathers and the other describing the features of bearing-down which may be deemed to aid expulsion of the egg.

In all these experiments a total of 42 birds were used of which 33 were laying hens, 5 non-laying hens, 2 immature hens and 2 were mature cocks.

(a) The reflex erection of the abdominal feathers

No technique of recording feather erection was evolved but the difference between the normal and the erect was quite clear, as can be seen from plate 1, and, furthermore, the actual movement of the feathers to the erect position could not be missed.

The feathers involved were the contour feathers surrounding the vent laterally and ventrally and extending for nearly half the length of the abdomen.

The stimulus. Feather erection was seen during oviposition and defaecation, the normal stimulus being, presumably, the stretching of the tissues by the egg or the faeces. Experimentally, it was observed when a balloon was used to distend the vagina or the rectum. This distension was not usually restricted solely to the respective viscus but distended part of the cloaca as well. Feather erection was not observed when a small balloon was inserted deeply into the vagina and inflated without producing any noticeable distension of the cloaca.

Touching the inner or outer surfaces of the cloaca was also an adequate stimulus.

Feather erection by either of these means was abolished by local anaesthesia of the cloaca or by deep general anaesthesia and it was observed in laying, non-laying and immature hens and in cocks.

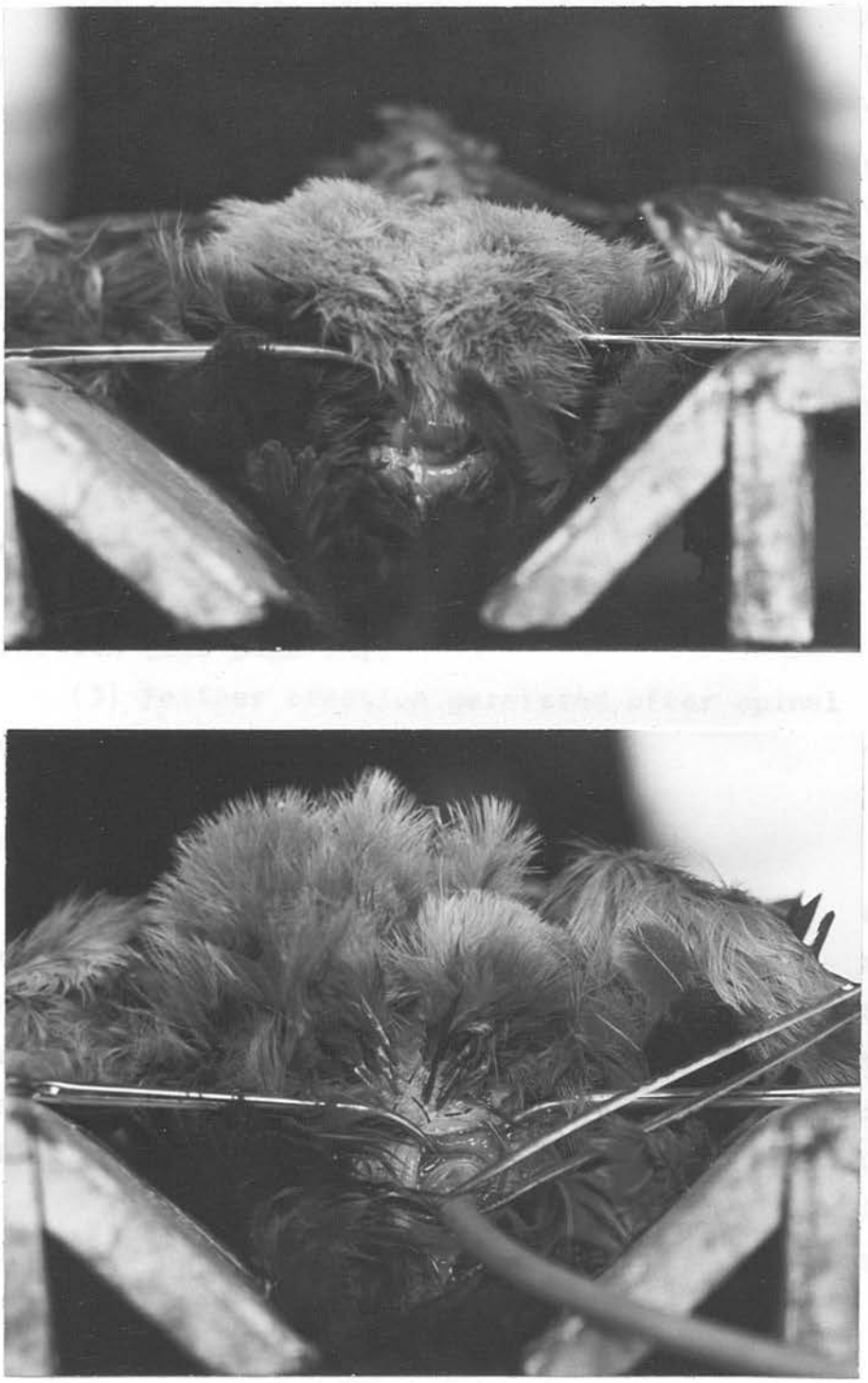


Plate 1: The position of the abdominal feathers before (above) and during (below) distension of the vagina and cloaca.

After spinal cord transection (T4-5) the reflex was no longer elicited in a hen and a cock when they were examined a short time after the operation (15-60 minutes) but in a second hen, examined for several hours afterwards, feather erection was constantly seen. In all 3 birds the success of the operation was verified post mortem.

Comment. The reasons for concluding that feather erection is a reflex separate from the bearing down reflex are as follows:-

(1) A tactile stimulus to the cloaca evoked feather erection alone.

(2) Bearing-down occurred without feather erection (see page 54).

(3) Feather erection persisted after spinal cord transection in one of the hens whereas bearing down was always abolished.

(b) Reflex bearing down.

Two features of this reflex gave themselves to graphic recording, the respiratory changes and the changes in abdominal pressure. The latter resulted from the combined contractions of the abdominal and respiratory muscles or from the respiratory muscles alone. Other features which could be observed, but which were not recorded, were the contracting abdominal muscles themselves and the expulsive force felt by the hand which inserted the balloon.

Respiration. There was an increase in both depth and frequency with the onset of bearing down (e.g. fig. 17). From the nature of the method the changes in the depth are only relative, rising on occasion to twice the resting value. For the frequency a mean figure was calculated from observations

on several birds; the resting frequency was 25.4 ± 1.38 per minute and the stimulated frequency 40 ± 1.69 per minute, a difference significant at the 0.1% level.

At times the respiratory frequency would be slower than normal (fig. 13); this occurred when the breath came in gasps during maximal bearing-down efforts.

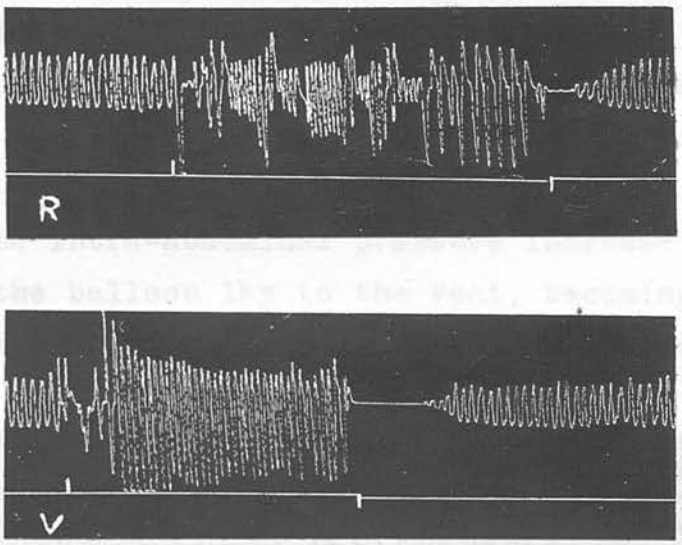


Fig. 13: Respiration showing (above) the effect of distension of the rectum and (below) the effect of distension of the vagina. Distension occurred between the vertical marks. Note the period of apnoea after removing the stimulus.
Time scale on this and the following figures given in fig. 17.

Periods of hyperpnoea during the operation of the reflex were often followed by a period of apnoea, a probable consequence of the previous over-ventilation (figs. 13 and 18).

Intra-abdominal pressure. The position of the balloon inside the abdomen affected its sensitivity to changes in pressure. Sometimes, as seen in figs.

17 and 19, the respiratory movements were recorded but it was always obvious when bearing-down began. When the respiratory movements were not recorded the tracing gave a very clear picture of the sharply "on" and "off" characteristic of the reflex; there was no tailing off. Another point to be noticed was that the abdominal pressure increased in steps corresponding to each increase in the size of the distending balloon (fig. 14). During this time the respiration maintained the same rate and amplitude as though this was an "all-or-none" response. Besides increasing with the greater vaginal distension, the intra-abdominal pressure increased the nearer the balloon lay to the vent, becoming maximal when the whole of the cloaca was distended.

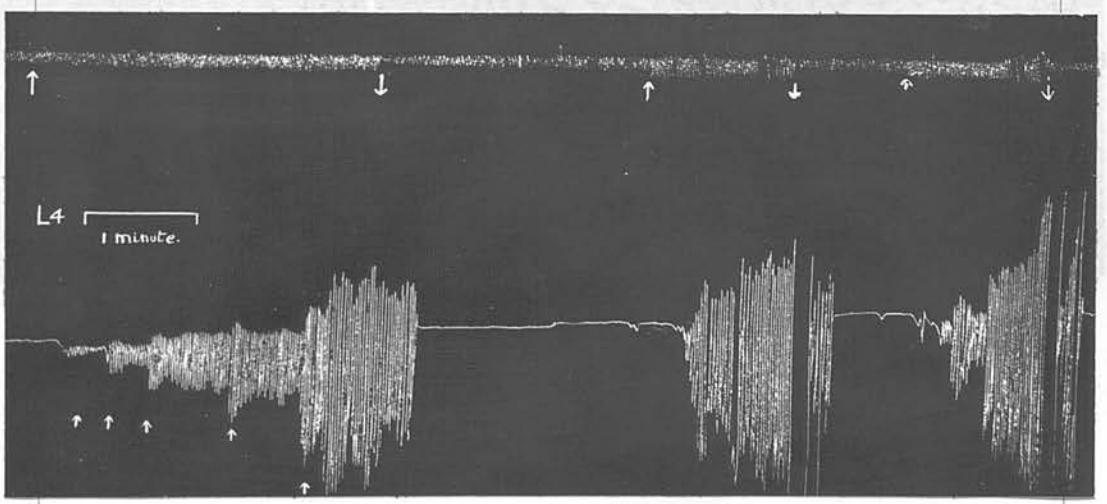


Fig. 14: Respiration (above) and abdominal pressure (below). ↑ indicates onset and ↓ indicates cessation of distension. The first period shows and effects of increasing distension.

The absolute pressure recorded during bearing-down ranged from 30 to 60 m.m.Hg.

The stimulus. Distension of the vagina and cloaca together always evoked the reflex. It was not sufficient merely to insert or withdraw a deflated balloon and the reflex was never evoked by a tactile stimulus.

Similarly, distension of the rectum and cloaca produced the same effects although there seemed to be less muscular effort compared with the bearing-down of vaginal origin.

Only the terminal part of the rectum was sensitive to the distending stimulus. When the balloon lay about 5 cms. inside the rectum there was no response no matter how much it was distended. This is

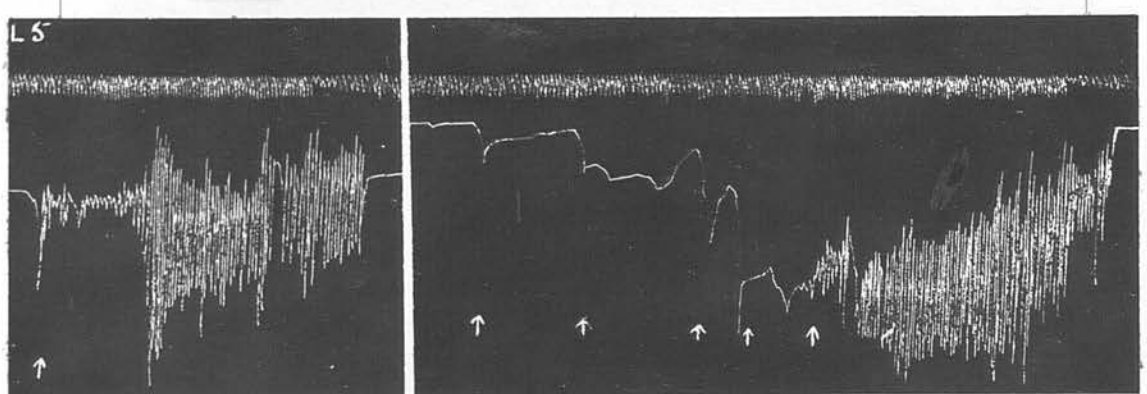


Fig. 15: Respiration (above) and abdominal pressure (below) of a non-laying hen. Left: distension of the vagina; right, distension of the rectum at the first arrow deeply and then more caudally at each succeeding arrow.

seen in fig. 15; the pressure in the recording balloon was affected by the increasing volume of the distending balloon but no rhythmical contractions ensued until the latter was withdrawn into a position nearer the anus.

It was not found possible to distend the cloaca alone without thereby distending part of the rectum and vagina but, on the other hand, by means of a

smaller balloon, it was possible to distend the cranial end of the vagina without producing any noticeable distension elsewhere. This purely vaginal stimulus always evoked bearing-down movements.

Local stretching of the vaginal wall was accomplished by pulling with artery forceps in such a way that there was no tension on the cloaca or on any other part of the vagina. This local stimulus was adequate to evoke all the features of the bearing-down reflex (but not erection of the feathers).

Finally, it was shown the distension of the uterus, by a balloon inserted per vaginam, did not cause bearing-down.

Birds. The reflex was studied mostly in laying hens but it was observed also in non-laying hens in which the vagina was reduced in size. In these birds the expulsive force appeared to be much less than in the laying birds. The response from the rectum was the same in both (figs. 13 and 16).

The rectal bearing-down reflex was demonstrated in 2 mature cocks.

In another experiment, 2 immature pullets aged 12 weeks were used to demonstrate the rectal reflex. Then, after a period of treatment with sex hormones (1 mg. oestradiol dipropionate per day for 6 days), which stimulated the growth of the oviduct, the vaginal reflex was observed. A very small balloon had to be used and although the hymen was thin by the completion of the hormone treatment, it was still intact and had to be broken. The response, though typical, was weak even compared with a non-laying hen. The weight of the vagina in these birds after treatment was 2.6 mg. compared with 4.5 gm. in a laying bird.

Abolition of the reflex. Local anaesthesia of the vagina and cloaca was accomplished within a few minutes by ½-1 ml. of Nupercaine or Procaine. It completely abolished the reflex from the vagina and rectum as shown in Fig. 16.

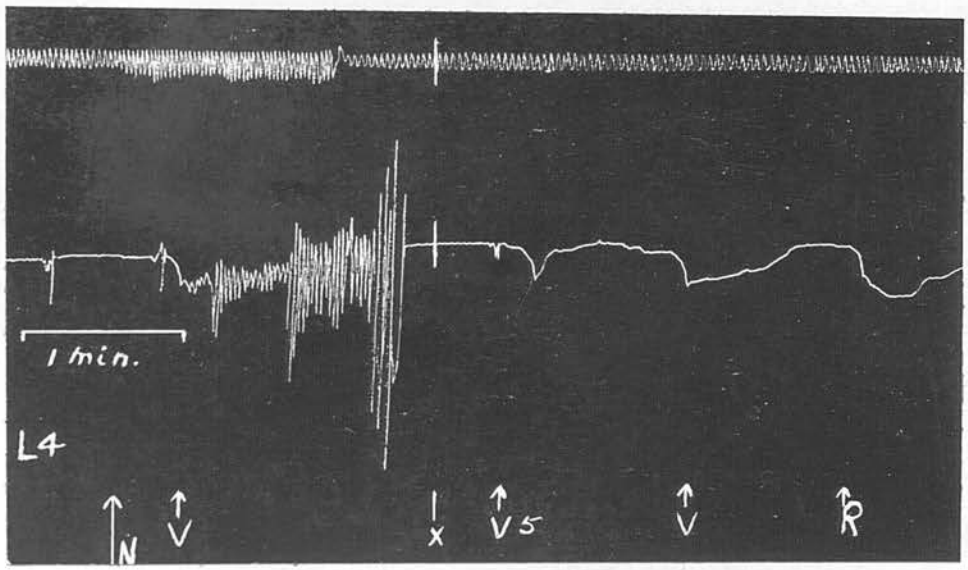


Fig. 16: Respiration (above) and abdominal pressure (below) showing the effects of rectal and vaginal distension after an injection of 1 ml. Nupercaine. ↑N = Nupercaine injected; ↑V = vaginal distension; ↑R = rectal distension; ↓=end of distension. At x the recording drum was stopped for 2 minutes.

Deep general anaesthesia by ether or phenobarbitone also abolished the reflex.

After spinal cord transection (T4-5) in 2 hens and 1 cock, there was no reflex bearing down in the period immediately following the operation or several hours later when recovery was complete.

Reflex bearing-down during oviposition. The conclusion to be drawn from the experiments so far is

that there exists a reflex arc having its sensory origin in the vagina (and rectum), which is stimulated by distension, and which, on the motor side, gives rise to the co-ordinated activity of the birds respiratory and abdominal muscles recognized as the bearing-down movements of oviposition (and defaecation).

By recording the changes in respiration and abdominal pressure which occur during oviposition it could be shown that there is no difference between the experimental induction of the reflex by means of balloons and its natural induction by means of an egg. That, in fact, the vaginal reflex functions as a normal part of oviposition and not, for instance, as an emergency mechanism or only in the presence of unnatural objects.

In fig. 17 the hen had just started to lay when

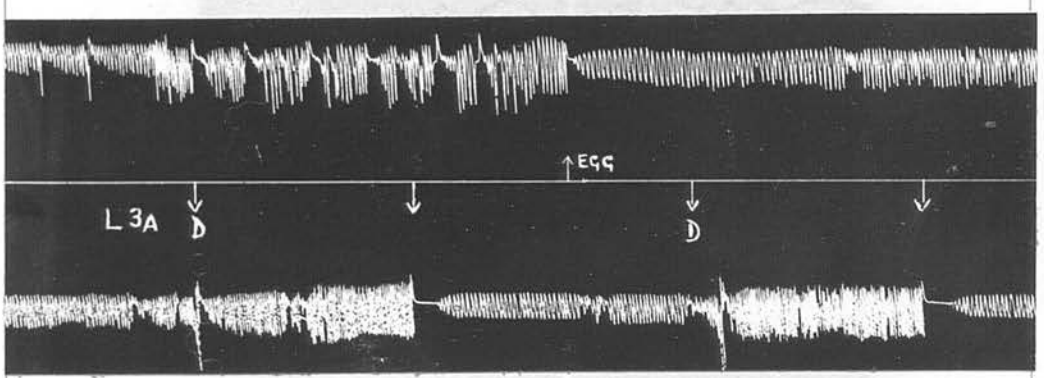


Fig. 17: Respiration. Top line shows the effect of normal oviposition terminating at ↑ and followed by a control period. Bottom line shows the effects of 2 periods of vaginal distension between ↓D and ↓ .

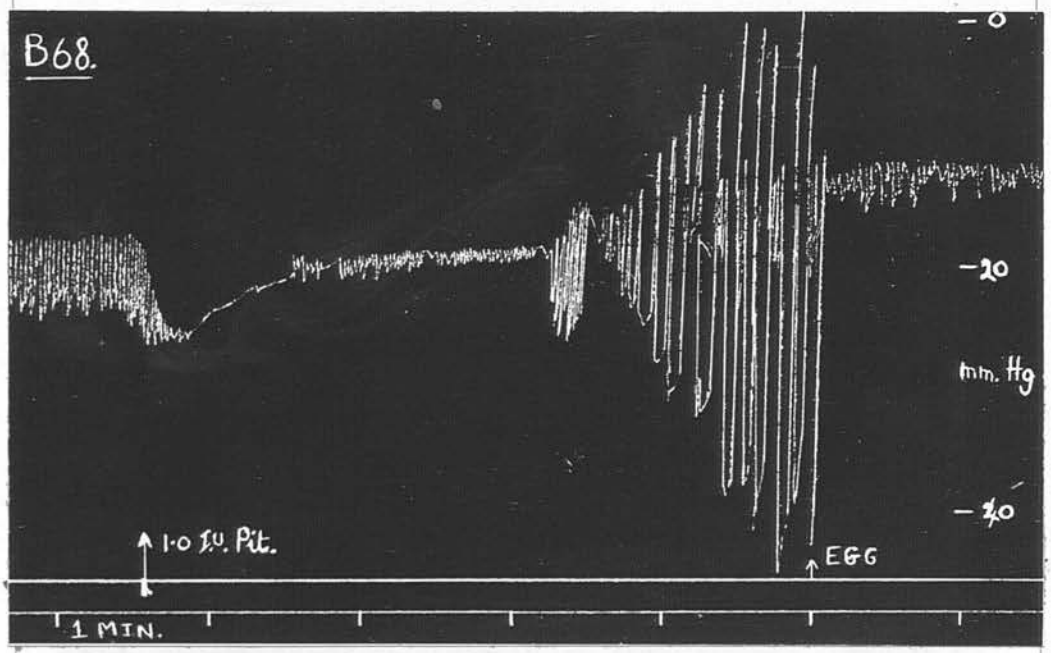


Fig.18: Abdominal pressure showing changes during induced oviposition.

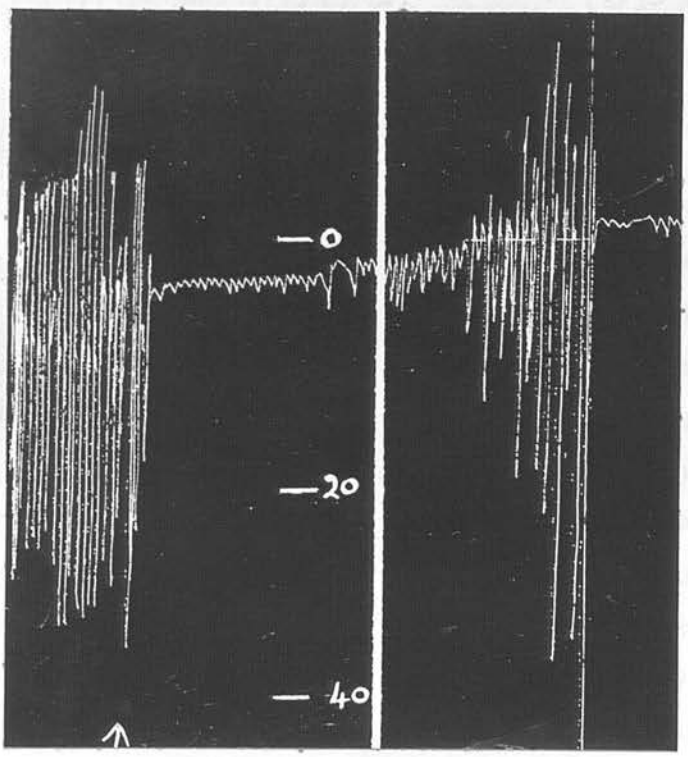


Fig.19: Abdominal pressure. Left: during oviposition; right: during distension of the vagina.

the tracheal cannula was attached. The tracing shows an increased frequency with deep breaths occurring in bouts rather like Cheyne-Stokes breathing and at the end of the longest of these bouts the egg was laid. There followed a short period of apnoea. On the lower line of this tracing there are 2 demonstrations of the respiratory changes following vaginal distension by a balloon. The absence of a rest between bouts of respiratory efforts and the greater period of apnoea afterwards are in contrast with the naturally occurring reflex changes. It is possible that these differences were caused by the volume of the balloon which in this case was greater than the egg. It might, therefore, have distended more of the vagina and brought about a supra-normal expulsive effort.

In fig. 19 the abdominal pressure is seen to be similar during oviposition and distension of the vagina.

The most complete record of the contractions of labour are seen in Fig. 18. The onset of normal oviposition had been prevented by the injection of 1 mg. adrenaline subcutaneously. At the start of the experiment 5 hours later, oviposition was induced by injecting 1 unit of pituitrin intravenously. The tracing shows that the normal respiratory movements were drastically suppressed after pituitrin was injected. The cause of this is not clear; it is unlikely that complete apnoea would persist for so long. About 2.5 minutes after the injection, bearing-down commenced and continued for two minutes until the egg was laid. The contractions were weak and frequent at first but gave rise to a series of

increasingly powerful efforts with respiratory gasps in between. The distance between pairs of lines on the lower half of the tracing indicates the periods of maximal effort during which the breath was held. It is interesting to note that of the 4.5 minutes which elapsed between the injection of pituitrin and the egg being laid, 2 minutes were spent in bearing-down leaving 2.5 minutes in which the drug reached the uterus and evoked the propulsion of the egg into the vagina.

A series of further experiments on these lines was undertaken to see whether or not oviposition would be impaired if the bearing down reflex was temporarily abolished by means of vaginal anaesthesia.

The scheme was as follows. On the morning of the experiment the bird was injected with 1 mg. adrenaline to prevent the uterine egg from being laid. Four to five hours later, using light ether anaesthesia for restraint, the bird was treated with one ml. of a local anaesthetic, injected into the tissues of the vagina and cloaca, and then with 1 unit of pituitrin (intravenously) to induce oviposition.

Initially Nupercaine was used but this was very long acting in the hen and tended to reinforce the general anaesthesia. As a result of this one bird died, the induced egg remaining in the vagina for 50 minutes up to the time of death. Another bird, treated with Nupercaine, retained an egg in its vagina for over 2 days; it was later removed manually.

The shorter acting Procaine was used for the main series of experiments and the results are given in Table 5. As would be expected, the egg could not be laid so long as reflex bearing-down was prevented by the anaesthetic. This delay lengthened the time

of oviposition from 5 minutes to 19 to 120 minutes. The sooner oviposition was induced after anaesthetizing the vagina the longer was the delay. The sum of columns 1, 3 and 4, that is the total time spent in laying less the time spent in bearing down, should be a measure of the duration of action of Procaine. Four of these totals fall within the range of 32-41 minutes, about the expected value, but 2 are well outside this range, 56 and 116 minutes in birds B4 and B3 respectively.

Table 5. The effect of vaginal anaesthesia on oviposition.

		Time in Minutes				
Bird No.		1 Procaine to Pituitrin	2 Pituitrin to Laying	3 Pituitrin to egg seen at vent	4 Difference in time between events in columns 3 and 5.	5 Start of bearing-down to laying
Controls	B64	-	8.0	7.0	0	1.0
	B65	-	7.0	5.5	0	1.5
	B68	-	4.0	2.0	0	2.0
	B69	-	4.0	1.5	0	2.5
	B70	-	3.0	1.5	0	1.5
	B5	-	7.0	4.0	0	3.0
	B6	-	2.0	1.0	0	1.0
Treated	B71	17	28	3	21	4
	B72	18	19	2	12	5
	B73	23	19	5	11	3
	B3	0*	120	3	113	4
	B4	6	54	2	48	4
	B7	10	30	3	24	3
Mean of Controls		-	5.0	3.2	0	1.8
Mean of Treated		14.8	45.0	3.0	38.2	3.8

*Natural induction of oviposition occurred in this bird shortly after the injection of Procaine.

The conclusion to be drawn from these experiments is that the bearing-down reflex plays an essential part in normal oviposition. Whether this conclusion is entirely justified will be considered in the discussion.

APPENDIX

A. Nesting behaviour (see page 9)

The oviduct was removed from 2 juvenile hens. When they were adult and in laying condition as judged by the appearance of the comb, the vent and the pubic bones, they were observed to visit the trapnests frequently. A record of the number of visits over a short period is given below; the figure '2' indicates that the bird was found twice in the nest on the same day.

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Bird A	1	-	1	2	-	2	1	-	1	1	-	1	1	1	1	1	1
Bird B	2	1	2	1	1	2	-	-	-	1	1	1	1	1	1	1	1

B. Avian pituitrin (see page 14)

The posterior lobe of the pituitary gland of groups of 10-20, 6 week old chicks was dissected out and prepared into a powder by a method outlined by Burn (1950). The Soxhlet extraction was omitted and the final solution was prepared by adding 2 ml. 0.25% acetic acid per mg. of powder instead of 1 ml. as in the standard method.

The ability of the extract to produce premature oviposition when injected intravenously was compared with commercial pituitrin. Prematurity was judged by the speed of the response after the injection, usually within 5 minutes and never later than 10 minutes, and by injecting hens which were not expected to lay until several hours later in the day. The activity of the extract was lost after treatment with NaOH; this is a test which distinguishes pituitrin from histamine which might be present.

		Positive	Negative
Pituitrin	1.0 I.U.	15	0
	0.5 I.U.	13	10
Extract	0.1 ml.	17	1

One I.U. pituitrin is equivalent to 0.5 mg. standard powder; 0.1 ml. of the avian extract is equivalent to 0.05 mg. of a similar powder. Therefore the extract was 10 x more potent than the commercial preparation.

C. Experimental premature oviposition (see page 17)

It was found by Huston & Nalbandov (1953) that a loop of suture thread inserted into the magnum wall in such a way as not to obstruct the lumen, inhibited egg laying. They interpreted their findings by proposing that the thread affected the production of ovulating hormone by stimulating nerves connecting the oviduct with the pituitary gland. If such a pathway exists then it might play a part in obtaining the synchronism that regulates oviposition and ovulation.

To meet certain objections the original experiment was repeated on the magnum and also extended to include the isthmus and the uterus. This work is not completed but the preliminary results demonstrate a new feature of the oviduct which may have some bearing on the mechanism of oviposition.

Table 6 shows the number of hard and soft shelled eggs laid after the treatment. Once egg production returned to normal the bird was removed from the experiment.

Table 6. The number of hard and soft shelled eggs laid after disturbance of the oviduct

Position of thread	Hen No.	Control		1-10 days		11-20 days		21-30 days		31-40 days		41-50 days		51-60 days		61-70 days	
		H	S	H	S	H	S	H	S	H	S	H	S	H	S	H	S
Magnum	1	8	0	2	0												
	2	9	0	1	4	0	2	8	2								
	3	5	0	0	0	6	1	8	0								
	4	7	0	0	1	8	0										
	5	7	0	2	3	6	0	8	0								
	6	10	0	1	1												
	7	7	0	4	0	0	5	2	4	7	1	4	1				
Isthmus	12	7	0	5	0	4	0	1	0	0	1	0	0	0	0		
	13	5	0	0	0	4	1	1	4	2	3	0	4	1	4		
Uterus	14	7	0	0	6	0	6	0	6	0	3	0	5	0	2		
	15	8	0	0	0	0	5	0	6	0	7	0	6	0	5	0	6
	16	7	0	1	2	0	4	1	5	4	1						
	17	7	0	2	3	0	3	0	4	0	2						
	18	4	0	1	4	0	4	0	6	0	4	0	8	0	3	0	4
	19	6	0	1	5	0	7	0	5	0	2	0	8	0	6	0	6
	20	5	0	0	2	0	6	0	5	0	5	0	7	0	5	0	3
	21	9	0	4	3	2	5	0	7	0	5	0	8	0	5	0	6
	Total	53	0	9	25	2	40	1	44	4	29	0	42	0	26	0	25
	Mean	6.6	0	1.1	3.1	0.25	5.0	0.13	5.5	0.5	3.6	0	7.0	0	4.3	0	5.0
	Mean H+S Eggs	6.6		4.3		5.3		5.6		4.1		7.0		4.3		5.0	

H = No. of hard shelled eggs
S = No. of soft shelled eggs
laid in 10 day periods

It will be seen that although egg production was drastically reduced in some of the birds, in many, production was almost unaffected but the eggs were all soft shelled. The birds with a uterine thread continued to lay soft shelled eggs for up to 20 days afterwards and three of them continued in this way when they resumed laying after a moult of nearly 3 months duration. Not all the birds which were treated gave this response. Not included in the table are 4 hens with a thread in the magnum, 6 with a thread in the uterus, 6 controls which had the uterus exposed and 3 which suffered more extensive trauma of the uterus or magnum. In all these, egg production continued normally.

The soft shelled eggs were not laid throughout the day but mainly in the night or early morning. In a sample of 20 eggs, 17 were laid between 21.00 and 9.00 and 3 between 15.00 and 17.00. It is thought that these eggs were laid as soon as they reached the uterus. It was impossible to locate them by palpation which should not have been the case had they been delayed rather than premature.

Many of these soft shelled eggs lacked membranes as well as a shell and, occasionally, a thin shelled egg would be laid.

D. Publications

Parts of this Thesis have been published as the following papers:

"Some observations on oviposition in the fowl" Quart. J. Exp. Physiol. 38, 61, (1953).

"Premature oviposition in the fowl" Nature 172, 1098, (1953).

"Reflex bearing-down in the hen" 10th World's Poultry Science Congress (1954), (In the press).

CONCLUSIONS

A. The motility of the uterus and vagina in vitro.

1. The longitudinal and circular muscles of the uterus do not differ in their motility and response to adrenaline.

2. All regions of the uterus show the same motility and response to adrenaline.

3. Adrenaline and pituitrin are antagonistic on the uterus.

4. The uterus of a non-laying hen shows weak but characteristic motility.

5. The vagina is refractory to pituitrin.

6. Adrenaline contracts the circular muscles of the vagina but is without effect on the longitudinal muscles.

7. The vagina of a non-laying hen is not motile.

8. The action of ephedrine on the uterus is unlike that of adrenaline; it consists of a rise in tone and the eventual extinction of all contractions.

9. Ephedrine on the vagina causes a rise in tone of the circular muscles similar to, but slower than, the effect of adrenaline.

10. Ephedrine on the isthmus causes a rapid rise in tone comparable to the effect of adrenaline.

11. Ephedrine at high concentrations can prevent the action of pituitrin.

B. The effect of adrenaline on oviposition

12. A dose of 1 mg. adrenaline delays oviposition at least 6 hours.

13. After 1 mg. adrenaline the sensitivity of the uterus to pituitrin is reduced for at least 4 hours.

14. Adrenaline lowers egg production by 20-100%

C. The role of the abdominal muscles in oviposition

15. Eversion of the uterus does not occur during oviposition.

16. Oviposition is difficult or impossible when the abdominal muscles are damaged.

17. Oviposition is initiated normally in the spinal hen (lesion T6-L6).

18. Oviposition is often protracted but nearly always accomplished in the spinal hen.

19. Ovulation is inhibited in the spinal hen.

20. Erection of the feathers surrounding the vent occurs after tactile stimulation of the cloaca.

21. Feather erection is shown by laying, non-laying and juvenile hens and by cocks.

22. Feather erection may persist after spinal cord transection (T6).

23. Distension of the rectum, vagina and cloaca causes reflex bearing-down in laying, non-laying and juvenile hens and in cocks.

24. Bearing-down consists of contractions of the abdominal muscles, which result in increased intra-abdominal pressure, and of increased depth and frequency of respiration.

25. Bearing-down during oviposition is similar to experimentally induced bearing-down

26. The bearing-down reflex may be abolished by local anaesthesia of the vagina and cloaca, deep general anaesthesia and spinal cord transection (T6).

27. Ejection of an egg from the vagina is not possible during local anaesthesia of the vagina.

Appendix.

28. Nesting behaviour does not depend on the presence of an oviduct.

29. Pituitrin may be extracted from the pituitary gland of immature hens in a more potent form than commercial preparations.

30. A loop of thread inserted into the wall of the uterus causes soft shelled eggs to be laid for up to 70 days afterwards.

DISCUSSION

In the review of the literature oviposition was considered in two parts, the movements of the egg and the factors controlling those movements. This division of the subject is also suitable for the discussion and will be followed after first considering the action of adrenaline on egg production.

Adrenaline and egg production

Egg production may be depressed by the administration of gonadotrophic hormones, (Pearl & Surface, 1912; Schoeller & Gehrke, 1933; Dunham & Riddle, 1942), by the injection of various non-specific substances such as extracts of tissues or casein (Fraps & Neher, 1945), by surgical operations (Rothchild & Fraps, 1945, 1947), and by fright (Stieve, 1918; Warren, 1930). In all these cases the ovarian follicles became atretic.

To this list must be added adrenaline but it cannot be said at this stage whether there is any common mechanism by which egg production is lowered. It is possible that adrenaline did not inhibit ovulation but, by its action on the oviduct, prevented the ovum from entering the infundibulum. This is considered unlikely since in 2 birds which were killed after an injection of adrenaline there were several atretic follicles and it may be presumed that ovulation was inhibited.

From what is known of the mammalian adrenosympathetic system it is possible that the various non-specific agents which interrupt egg production, such as surgical trauma or fright, act by promoting the release of adrenaline but this hypothesis would

stand on firmer ground if it were known that the birds adrenal gland responds in this way.

If adrenaline can inhibit ovulation then it might do so either by preventing the release of gonadotrophins from the pituitary gland or by interfering with the metabolism of the follicle.

Perry (1941) has shown that adrenaline causes regression of the sparrow's gonads in the spring and that this effect was not modified by injecting gonadotrophins at the same time. This might mean that the gonads were the site of the adrenaline action but the dose that was used was only just sub-toxic and injections were continued for several days. Lyman (1942) and Kar & Ghosh (1952), using the pigeon, have shown that with moderate doses of adrenaline testis regression may be counteracted by the administration of gonadotrophins. Since the 2 mg. dose of adrenaline could prevent an ovulation that was imminent at the time of injection, and therefore outside the control of the pituitary gland, it would appear that the site of action might be directly on the ovary. Kraus (1947) has shown that the follicular wall contains smooth muscle cells which may assist the extrusion of the ovum; it is possible that they were inhibited by adrenaline.

The control of oviposition

Initiation. It was pointed out on page that the principle problem of oviposition was the nature of the initial stimulus. Three general types of agent exist; nerves, hormones and the spontaneous irritability of the oviduct. In the experiments on spinal birds the majority of eggs were expelled from a uterus which, at the time, was unlikely to have had

any nervous connection with the brain. Therefore, unless reflex arcs below the lesion were essential, the influence of nerves may be ruled out. A possible objection to this conclusion might be that by the time of the operation some essential nervous function (for instance sensory impulses from the uterus) may have been performed already, but on the other hand, 3 of the 9 eggs were ovulated, formed and laid while the bird was paraplegic.

It is well known in mammals that parturition does not depend on the presence of an intact nervous connection between the uterus and the brain (Marshall & Moir, 1952) and it has been stated that nearly all reproductive functions can proceed in the absence of the nerves supplying the reproductive organs (Reynolds, 1949).

The evidence that parturition might be initiated by hormones is inconclusive but there is enough to suggest that the oxcytocin theory of Dixon & Marshall (1924) is not without foundation. The hyphysectomy experiments of Smith (1932) and Allen & Wiles (1932), which showed that the posterior lobe was dispensible, were done before it was known that the hypothalmus can secrete oxytocin. Later work has shown that cats and guinea pigs suffering from hypothalamic lesions may experience severe dystocia during parturition (Fisher, Magoun & Ranson, 1938; Dey, Fisher & Ranson, 1941) and a mechanism has been described whereby the uterus itself reflexly stimulates the release of oxytocin (Ferguson, 1941).

Parturition is a complex event and, as Reynolds (1949) has pointed out, it is incorrect to talk of a single cause when there are so many contributory factors. The experiments in which premature oviposition

was evoked by the presence of a thread in the uterus, suggest that increased uterine irritability may be enough to promote expulsion of the egg and if oxytocin is involved in normal laying it may be in the capacity as an adjuvant.

The conclusion that the uterine thread stimulated uterine motility is only tentative but it is the simplest explanation of the results and finds some support from various experiments on mammals. Marshall & Moir (1952) state that a foreign body in the uterus causes contractions and Carlton & Philps (1933), found that loops of thread were as effective as Gräfenburg rings in preventing implantation in rabbits. Lauffer & Reynolds (1938) showed that, in the absence of oestrogen, uterine motility could be induced only when there was an inflammation of the myometrium. Lastly, according to the "law" of Alvarez (Alvarez & Hosoi, 1929), an irritation at any point of the intestine tends to slow the progress of material coming from the stomach and to hasten the progress of material caudal to the irritation.

These papers show that smooth muscle responds to a local irritation by increased motility and it is possible that the same conclusion applies to the smooth muscle of the oviduct.

Adrenaline and delayed oviposition. There seems little doubt that adrenaline delays oviposition by means of its inhibitory action on the uterus. It has been shown that there is no differentiation between regions or muscle layers in their activity and reactivity *in vitro* and Morash & Gibbs (1929) have reported that adrenaline inhibits the oviduct *in vivo*. Furthermore it has been shown that the reactivity of the uterus to pituitrin is reduced by adrenaline both *in vitro*

and in the intact bird.

The decline in sensitivity to pituitrin which was observed *in vivo* lasted for at least 4 hours and this compares favourably with the delay in the time of oviposition which was at least 6 hours. The dose of pituitrin that was used was probably more than the minimum required since the movements of oviposition appeared somewhat exaggerated compared with normal. Therefore, there is some evidence that the effects of adrenaline persist long enough to account for the observed delay. But in some cases the egg was held in the uterus for 12-48 hours, that is for long after the effects of adrenaline should have disappeared. This again raises the question of the nature of the primary motor stimulus to oviposition. There may be a mechanism which works only periodically and although uterine sensitivity may be restored the egg cannot be expelled until the next stimulus arrives. On the other hand, the uterus may be continuously expulsive after a certain time and remain so as long as the egg remains there. Thus as soon as the effects of adrenaline disappear the egg would be laid. Closer attention to the time of laying after a delay would help to clear up this point. It will be recalled that after a delay of a different kind (by removal of the post ovulatory follicle) the time of oviposition was influenced by the diurnal light rhythm (Rothchild & Fraps, 1944b), which suggests that there may be a periodic rather than a continuous mechanism.

The functional significance of the action of adrenaline may be that of allowing the bird to withhold the egg should circumstances be unfavourable at the normal time of oviposition. If this interpreta-

tion is correct then it presumes that the bird's adreno-sympathetic system behaves in essentially the same manner as the mammal's, a presumption that is not at present justified. To be on firmer ground, adrenaline should be detected in the blood in the requisite amount after a fright or similar disturbance which would cause oviposition to be delayed.

The reaction of the pregnant uterus of mammals to adrenaline, with which this work on the hen may be compared, varies from species to species and, in some, changes during pregnancy. The uterus of the rat, mouse, cow, sow and ewe is inhibited at the end of pregnancy while the uterus of the cat, bitch, doe and woman, unlike the hen, is stimulated (Reynolds, 1949). It does not follow that because the uterus is stimulated that adrenaline thereby promotes expulsion of the foetus. Kaiser & Harris (1950) have shown that the force of the labour contractions in woman is lowered by adrenaline, a finding which agrees with the view that difficulties in childbirth are often caused by emotional stimulation of the adreno-sympathetic system.

Bonnycastle & Ferguson (1941) showed that the motor action of adrenaline on the rabbit's uterus produced contractions unlike those of normal labour and that, by contracting the cervical segment more than the placental segment, the expulsion of the foetus was prevented.

It may be then, that the species differences in the reaction of the pregnant uterus to adrenaline are more apparent than real and that the final result, whether "motor" or "inhibitor", is always anti-expulsive.

The inhibitory action of adrenaline has been a useful tool in the study of the bearing-down reflex by allowing the time of (induced) oviposition to be controlled; it might be useful as a means of delaying

oviposition in birds with a uterine thread so as to learn whether shell secretion may still occur.

The action of ephedrine. Weiss & Sturkie (1952) explained the action of ephedrine on oviposition in terms of the inhibition of uterine motility like that caused by adrenaline. But it has been seen that ephedrine does not inhibit the uterus *in vitro* and that concentrations of the order of 10^{3} are required before the reactivity of the uterus to pituitrin is reduced. This concentration is achieved by a dose of ephedrine only 10 times as small as that required to inhibit ovulation in the whole bird. It may be, then, that ephedrine does not act on the uterus or that its behaviour *in vitro* is no guide to its behaviour *in vivo*. It is well known that ephedrine differs from adrenaline in many respects (Sollman, 1948) and if the suggestioniis correct that it owes its adrenergic properties to its ability to inhibit the enzymes which inactivate adrenaline (Gaddum & Kwiatkowski, 1939), then a direct action on the isolated uterine muscle would not be expected.

The movement of the egg

The motility of the vagina. The finding that the vagina of the hen differs from the uterus in its motility and response to drugs agrees with work on the mammalian vagina. Gunn & Franklin, (1923), Runge, Hutter & Wittmann, (1939), Custo, (1939), Genell, (1939), and Dworzak, (1939), all report that the vagina besides showing comparatively weak spontaneous contractions, is contracted by adrenaline and is refractory or less sensitive to pituitrin. In contrast with the fowl, Gunn & Franklin (1923) state that the circular and longitudinal muscles of the rab-

bit both give a motor response to adrenaline.

The insensitivity of the vagina to pituitrin would be expected on mechanical principles, otherwise, when inducing oviposition by pituitrin, the egg might find it impossible to enter a vagina that had already contracted.

The functional aspects of the response to adrenaline are obscure. If it were anti-expulsive then it might be expected that the bearing-down reflex would be inhibited as well otherwise the vaginal muscles and the abdominal muscles would be working against each other. An expulsive action would seem more likely especially as there are no reports of eggs being stored in the vagina. The differential response of the muscles may result in the egg being squeezed out of the vagina like paste out of a plastic tube; the circular muscles contracting while the longitudinal ones "stand firm". This argument presupposes that the vaginal muscles are used during oviposition and also that endogenous adrenaline would have time to function in the 1-2 minutes that the egg is in the vagina.

It should be remembered that the vagina is the pathway for sperm transport and it has been stated (Lake, personal communication) that sometimes, during artificial insemination, sperm are ejected from the vagina and consequently few fertile eggs are laid. This may be a result of fright during the insemination which causes an adrenaline-induced contraction of the vaginal muscles.

Reflex feather erection. The conclusion that this is a separate reflex from bearing-down rests mainly on the fact that feather-erection could be

evoked independently of bearing-down by a different type of stimulus (tactile), and, conversely, that bearing-down could occur in the absence of feather erection when the cranial end of the vagina was distended. The evidence from the spinal hens, though suggestive, is inconclusive.

Another type of local feather erection is seen in angry hens in which the hackles are raised, a phenomenon which recalls the pilo-motor reaction of angry cats and dogs. Dukes (1947) states that both hair and feather ruffling occur after the injection of adrenaline though he does not distinguish between a local response, as shown by the abdominal or neck feathers, and a general response, as seen in dust bathing or during cold weather when all the body feathers are ruffled.

Two points arise when considering the possible benefits which may be derived from abdominal feather erection.

The first is that without this mechanism, the abdominal feathers would soon become soiled by faeces and loose their value as thermal insulators. It is, in fact, remarkable how clean the feathers around the vent remain in most hens.

Secondly, the transference of sperm from cock to hen would appear to be hindered by the feathers which normally completely cover the vent of both birds and it is possible that the tactile stimulus of mating would cause the feathers to be moved out of the way.

Reflex bearing-down. It is generally known

by obstetricians that the abdominal contractions of the second stage of labour are "automatic" and it is stated by Beck (1951), in a standard work on the subject, that these contractions are due to a reflex which is inaugurated by the pressure of the foetus on the pelvic floor.

The clinical evidence for the reflex nature of bearing-down is supported by the few observations which have been made on experimental animals. Rudolph & Ivy (1930), in a paper describing the mechanism of parturition in the bitch, state that when a foetus entered the vagina, the abdominal muscles and diaphragm contracted but that the final expulsion of the foetus was caused by contractions of the vagina. Bearing-down was abolished by thoracic cord transection. In a post-partum bitch, the insertion of a vaginal balloon stimulated the respiration and increased the movements of the uterus; section of the nervi erigens abolished the former effect but not the latter.

The existence of a, presumably, nervous connection between the vagina and the uterus, by means of which uterine activity is increased, was first shown by Dembo (1885) and later by Ferguson (1941). This latter author showed in the rabbit and the cat that distension of the vagina evoked bearing-down efforts and a biphasic response in the uterus. This response consists of a period of increased frequency followed by a longer one of decreased frequency similar to the effect of stimulation of the hypogastric nerve (Schofield, 1952). Bearing-down, but not the uterine response, was abolished by spinal cord section (T 12). Ferguson's explanation of the biphasic response is that it is anti-expulsive and thereby delays the entry of a second foetus into the vagina before the first has been expelled. It would be interesting

to learn whether this vaginal-uterine reflex exists in monotocous mammals and in the hen or whether it has a different phylogenetic history from the bearing-down reflex.

The final reference to paper dealing with bearing-down is that by Franklin (in the press) in which he describes an increased blood pressure and changes in posture in rabbits as well as contractions of the abdominal muscles and diaphragm on distending the "narrow region of the birth canal" (vagina?).

It may be concluded that the bearing-down reflex is essentially the same in the fowl, some laboratory mammals and in man.

The value of the reflex is obviously to increase the total expulsive force on the vaginal contents at the time of parturition. In man, where the foetus is large and the vagina short, bearing-down augments the uterine muscles. Besides increasing the abdominal pressure, the increased respiration would have the effect of circumventing the normal panting mechanism which would operate only after a period of muscular work when the CO_2 tension in the blood had risen. This might delay the onset of muscular exhaustion especially in long labours.

There is no evidence that the bearing-down reflex is under voluntary control. Defaecation, a similar reflex, may be controlled in many mammals and Tinbergen, (1953) suggests that the herring gull may do so. It should be remembered however, that the opportunity for learning to control defaecation occurs several times daily from birth whereas bearing-down would occur relatively infrequently and only at maturity.

The role of the abdominal muscles and the bearing-down reflex in normal oviposition and parturition.

A reflex arc consists of sense organs, sensory and motor nerves and effector organs. By destroying

the function of one of these parts during oviposition the reflex as a whole should be abolished and its dispensibility shown.

In the experiments involving anaesthesia of the vaginal sense organs, it was seen that oviposition was prevented for as long as the anaesthesia lasted. The conclusion is, therefore, that reflex activation of the abdominal muscles is essential.

The observations at laparotomy suggested that the abdominal muscles, the effectors of the reflex arc, were essential for normal oviposition since when they were damaged oviposition was either delayed or impossible.

It was shown that section of the spinal cord abolished reflex bearing-down in acute experiments and therefore it would be expected that, with the link between the sensory and motor organs severed, oviposition in the spinal hen would be impossible. But it was seen that although oviposition was often protracted, it was always finally accomplished; only one out of 11 eggs was not delivered.

Taking the higher mammals as a whole, there appears to be a similar discrepancy. It is well known that human paraplegics can deliver their young and also spinal dogs and cats in which the completeness of the cord section was verified post mortem. This implies that successful delivery can be accomplished without the bearing-down reflex.

On the other hand, there is much evidence to show that the abdominal muscles are essential for the natural termination of labour. Beck (1951), states that forceps are often needed with paraplegics and always employed when spinal analgesia is used; also Marshall & Moir (1952) conclude that the vagina

is not expulsive and that the abdominal muscles must be used. Simpson's (1871) experiment on spinal sows is a classic in this field. He showed that parturition was initiated normally in the spinal sow and all except the last piglet of the litter was successfully delivered. The last one remained lodged in the vagina since the abdominal muscles were paralysed and there was no further piglet to transmit the force ofuuterine contractions to it. This last piglet is comparable to the egg laid by spinal bird "N" in the present experiments.

Lastly, Reynolds (1949), expanding on the work of Haughton (1873), has calculated that the human uterus never develops sufficient force to expel the foetus through the pelvis. It can dilate the cervix and rupture the membranes but the force necessary to deliver the foetus is greater than the force which the uterus can generate and the abdominal muscles must be used if delivery is to occur unaided.

It appears then that the abdominal muscles are essential but the reflex, which <u>normally</u> controls the muscles, is not. How then are the muscles controlled in the spinal animal? Perhaps by intact reflexes which form part of the mass reflex of Head & Riddoch (1918) as seen in the recovery stage of a spinal animal. It is well known that the evacuation of the bowel and the bladder becomes less incontinent as the animal recovers from spinal shock and that the abdominal muscles become functional again and it may be that this is what happens in cases of successful delivery after cord transection. Simpson's pigs, human cases of spinal analgesia, and the hen during vaginal anaesthesia, all suffer from an acute loss of the bearing-

down reflex while in cases of chronic clinical and experimental paraplegia the emergency mechanism has been put into use. In the hen, those showing the most protracted labour were those which started to lay shortly after cord transection; those laying later might have recovered from spinal shock.

SUMMARY

The physiology of oviposition in the fowl is reviewed.

Experiments on the uterus and vagina *in vitro* show that the vagina is refractory to pituitrin and shows an expulsive reaction to adrenaline while the uterus is sensitive to pituitrin and is inhibited by adrenaline.

From experiments with ephedrine it is concluded that the complex action of this drug makes it impossible to determine its physiological activity by purely *in vitro* tests.

Adrenaline is shown to have the ability to delay oviposition and to inhibit ovulation.

A reflex is described from the vagina which evokes bearing-down movements of the abdominal muscles and stimulates respiration. From experiments involving abolition of the reflex and from observations on laparotomized hens it is concluded that reflex stimulation of the abdominal muscles is essential for oviposition but experiments on spinal hens contradict this conclusion. In the discussion a similar discrepancy is shown to exist in mammals and an explanation is offered to account for it.

Reflex erection of the feathers surrounding the vent is described.

In the Appendix some experiments on the response of the oviduct to an irritant are reported.

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Addendum

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